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Cain

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(54) **METHOD FOR PRODUCING PROTEIN**

FOREIGN PATENT DOCUMENTS

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 50 days.

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(21) Appl. No.: **14/445,790**

Mezchrani, et al, "Manipulation of oxidative protein folding an PDI redox state in mammalian cells," The Embo Journal, Nov. 15, 2001, LNKD-PUBMED:11707400, vol. 20, No. 22, pp. 6288-6296.

(22) Filed: **Jul. 29, 2014**

Yoshida, H., et al, "XBPI mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor," Cell, Cell Press, Cambridge, MA, US LNKD-DOI:10.1016/S0092-8674(01)00611-0, vol. 107, Dec. 28, 2001, pp. 881-891.

(65) **Prior Publication Data**

US 2014/0356911 A1 Dec. 4, 2014

Lee, Ann-Hwee, et al, "XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response," Molecular and Cellular Biology, Nov. 2003, LNKD-PUBMED:14559994, vol. 23, No. 21, pp. 7448-7459.

Related U.S. Application Data

(63) Continuation-in-part of application No. 13/148,740, filed as application No. PCT/GB2010/000234 on Feb. 10, 2010, now Pat. No. 8,828,719.

Gunn, K.E., et al, "A role for the unfolded protein response in optimizing antibody secretion", Molecular Immunology, Pergamon, GB LNKD-DOI:10.1016/J.MOLIMM.2004.04.023, vol. 41, Jun. 5, 2004, pp. 919-927.

(30) **Foreign Application Priority Data**

Feb. 10, 2009 (GB) 0902180.9

Tigges, M., et al, "Xbp1-based engineering of secretory capacity enhances the productivity of Chinese hamster ovary cells," Metabolic Engineering, Academic Press, US LNKD-DOI:10.1016/J.YMBEN.2006.01.006, vol. 8, No. 3, May 1, 2006, pp. 264-272.

(51) **Int. Cl.**

C07K 16/00 (2006.01)

C12N 5/071 (2010.01)

Primary Examiner — Addison D Ault

(52) **U.S. Cl.**

CPC **C07K 16/00** (2013.01); **C12N 5/0682** (2013.01); **C07K 2317/14** (2013.01); **C12N 2510/00** (2013.01)

(74) *Attorney, Agent, or Firm* — McDonnell Boehnen Hulbert & Berghoff LLP

(58) **Field of Classification Search**

None

See application file for complete search history.

(57) **ABSTRACT**

The present invention relates to a recombinant host cell, wherein the cell is modified to increase the expression levels of Ero1 and XBP1 relative to the expression levels of Ero1 and XBP1 in an unmodified cell. The present invention also relates to a method of producing a recombinant protein of interest comprising expressing the recombinant protein of interest in the recombinant host cell.

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8 Claims, 24 Drawing Sheets

Figure 1

(a) Amino acid sequence of the spliced form of human XBP1 (SEQ ID NO: 1)

MVVVAAAPNPADGTPKVL LLSGQPASAAGAPAGQALPLMVPAQRGASPEAASGGLPQARKR
QRLTHLSPEEKALRRKLN RVAAQTARDRKKARMSELEQQVVDLEENQKLLLENQLLREKTH
GLVVENQELRQRLGMDALVAEEAEAKGNEVRPVAGSAESAAGAGPVVTPPEHLPMDSGGID
SSDSSEDILLILDNLDPMFFKCPSPPEASLEELPEVYPEGPSSLPASLSLSVGTSSAKLEAINE
LIRFDHIYTKPLVLEIPSETESQANVVVKIEEAPLSPSENDHPEFIVSVKEEPVEDDLVPELGISNL
LSSSHCPKPSSCLLDAYSDCGYGGSLSFPSDMSSLLGVNHSWEDTFANELFPQLISV*

(b) Nucleic acid sequence of the spliced form of human XBP1 (SEQ ID NO: 2)

atggtggtggtggcagccgcgcgaacccggcgacgggacccctaaagtctgtctgtcggggcagccgcctccgcgcgg
agccccggcgccaggccctgccgctcatggtgccagcccagagagggggccagcccgaggcagcgagcggggggctgccc
caggcgcgcaagcgacagcgccctcacgcacctgagccccgaggagaaggcgctgaggaggaaactgaaaaacagagtagca
gctcagactgccagagatcgaaaagaaggctcgaatgagtgaactggaacagcaagtggtagattagaagaagagaacccaaa
acttttgctagaaaatcagcttttacgagagaaaactcatggcctgtagttgagaaccaggagttaagacagcgctggggatggatg
ccctgggtgctgaagaggaggcggaagccaaggggaatgaagtgaaggccagtgccgggtctgctgagtcgcagcaggtgcag
gccagttgtcaccctccagaacatctcccatggattctggcggtattgactctcagattcagagtcgatctctgttggcattctgg
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ccagttccttaccagcctcccttctctgctcagtggggacgctcatcagccaagctggaagccattaatgaactaattcgtttgaccacata
tataccaagcccctagtcttagagataccctctgagacagagagccaagctaattgtgtagtgaaaaatcgagggaagcacctctcagc
ccctcagagaatgatcacctgaattcattgtctcagtgaaaggaagaacctgtagaagatgacctgtccggagctgggtatctcaaa
tctgtttcatccagccactgccc aaagccatcttctgctactggatgcttacagtgaactgtgatacgggggtcccttccccattcag
tgacatgtcctctctgctgtgtgtaaacattcttggaggacacttttgccaatgaactcttccccagctgattagtgctaa

Figure 2

(b) Amino acid sequence of human Ero1alpha (SEQ ID NO: 3)

MGRGWGFLFGLLGAVWLLSSGHGEEQPETAARCFQCQVSGYLDCTCDVETIDRFNNYRLF
PRLQKLLSDYFRYYKVNLRPCPFWNDSQCGRRDCAVKPCQSDEVPDGIKSASYKYSEEAN
NLIEECEQAERLGAVDESLSEETQKAVLQWTKHDDSSDNFCEADDIQSPEAEYVDLLNPERY
TG YKGPDAWKIWNVIYEENCFKPQTIKRPLNPLASGQGTSEENTFYSWLEGLCVEKRAFYRLIS
GLHASINVHLSARYLLQETWLEKKWGHNITEFQQRFDGILTEGEGPRRLKNLYFLYLIELRALSK
VLPFFERPDFQLFTGNKIQDEENKMILLEILHEIKSFPLHFDETSFFAGDKKEAHKLKEDFRLHF
RNI SRIMDCVGCFCRLWGNLQTQGLGTALKILFSEKLIANMPESGPSYEFHLTRQEIVSLFNAF
GRISTSVKELENFRNLLQNIH*

(b) Nucleic acid sequence of human Ero1alpha (SEQ ID NO: 4)

atgggcccggcggtgggattctgtttggcctcctgggcgcctgtggctgctcagctcgggccacggagaggagcagccccggag
acacgggcacagagggtgcttctgccaggttagtggttacttggaatgattgacctgtgattgaaaccattgatagatttaataactacag
gctttccaagactacaaaaacttctgaaagtgaactttaggtattacaaggtaaacctgaagaggccgtgtcctttcggaaatgac
atcagccagatgtggaagaagggaactgtgtctgtaaacatctgatgaagttcctgatggaattaaatctcgagctacaagta
ttctgaagaagccaataatctcattgaagaatgtgaacaagctgaacgactggagcagtggaatctctgagtgaggaaacaca
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ctctgtgtagaaaaagagcattctacagacttatctggcctacatgcaagcattaatgtgcatttgagtgaagatatctttacaaga
gacctggtagaaaaagaatggggacacaacattacagaattcaacagcgatttgatggaatttgactgaaggagaaggccaag
aaggcttaagaactgtatttctctactaatagaactaagggttatccaaagtgtaccattctcgagcgccagatttcaactctta
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tcatttttgctggggataaaaaagaagcacacaaactaaaggaggacttgcagctgatttgaaatattcaagaattatggattgtgt
tgggtgtttaaatgtcgtctgtgggaaatctcagactcagggttgggcactgctctgaagatcttatttctgagaaatgatagcaaat
atgccagaaatggacctagttatgagttccatctaaccagacaagaatagtatcattattcaacgcatttgaagaatttctacaagt
gtgaagaattagaaaactcaggaactgtacagaatattcattaa

Figure 3a

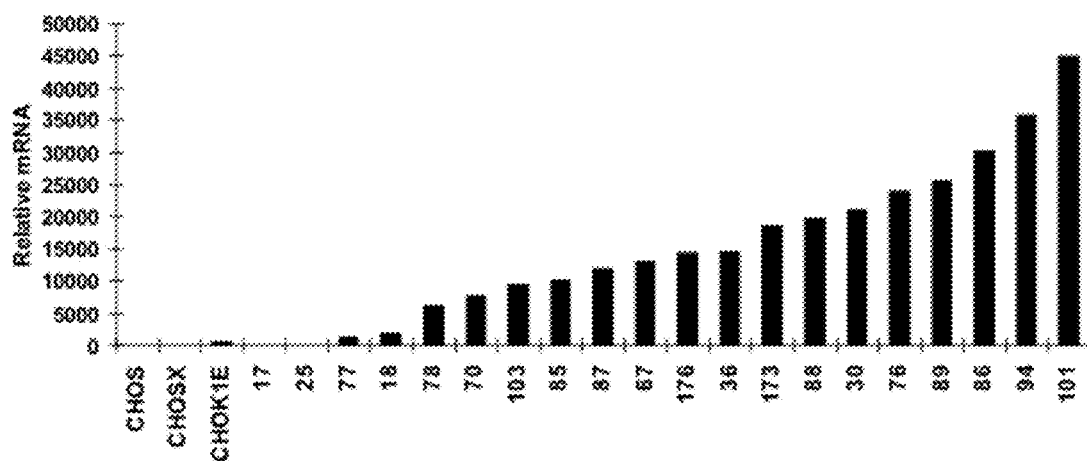


Figure 3b

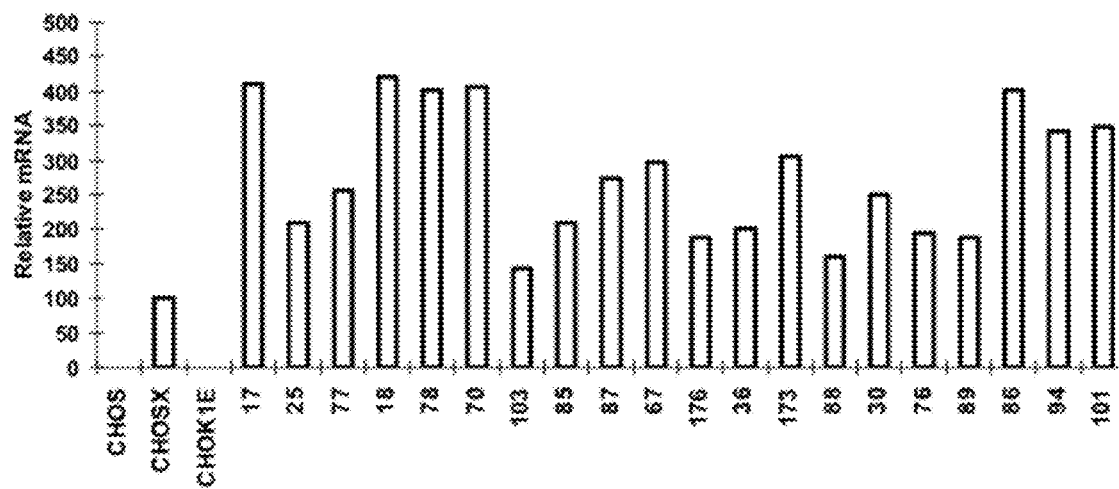


Figure 4

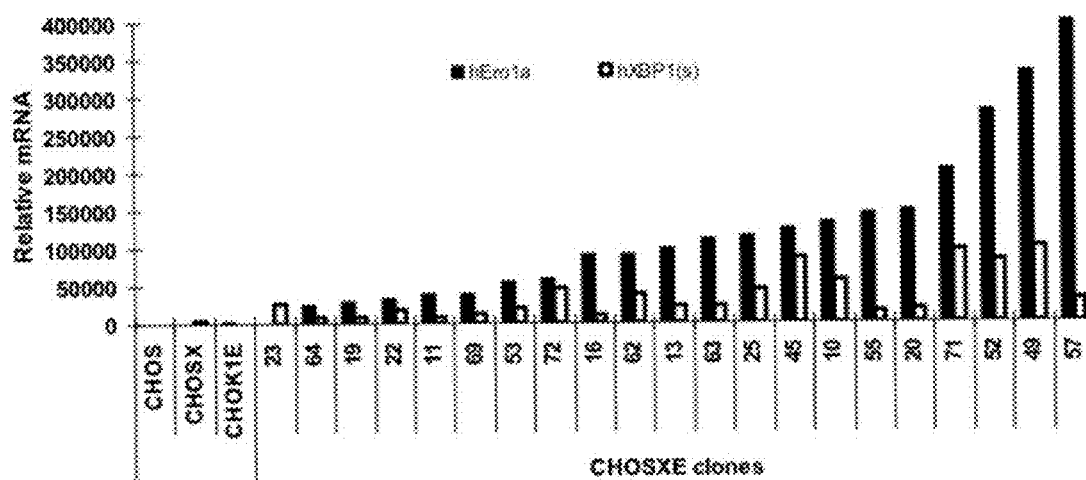


Figure 5

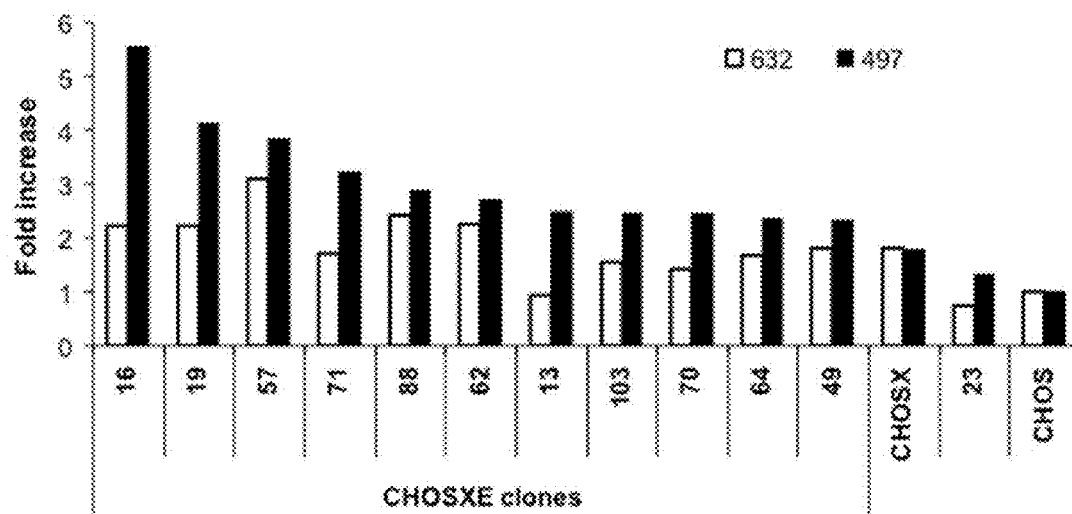


Figure 6

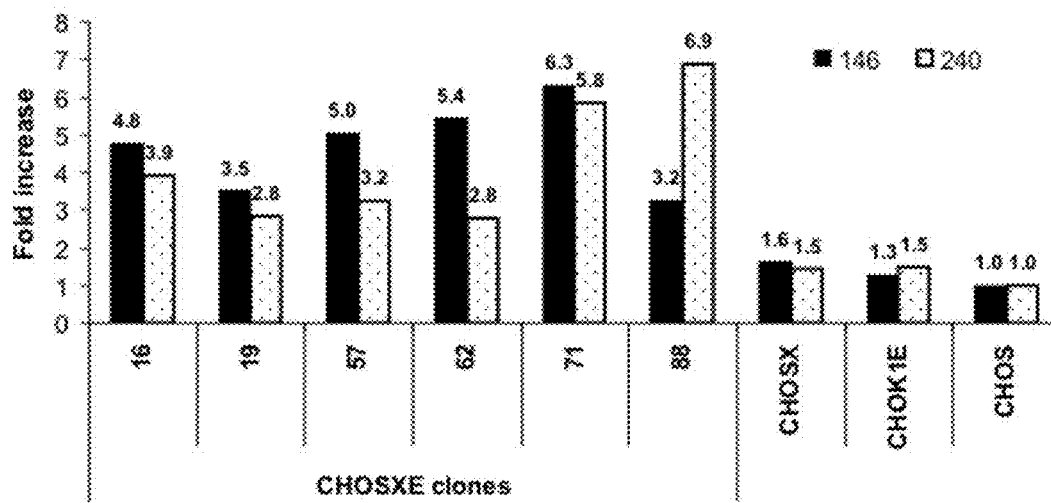


Figure 7

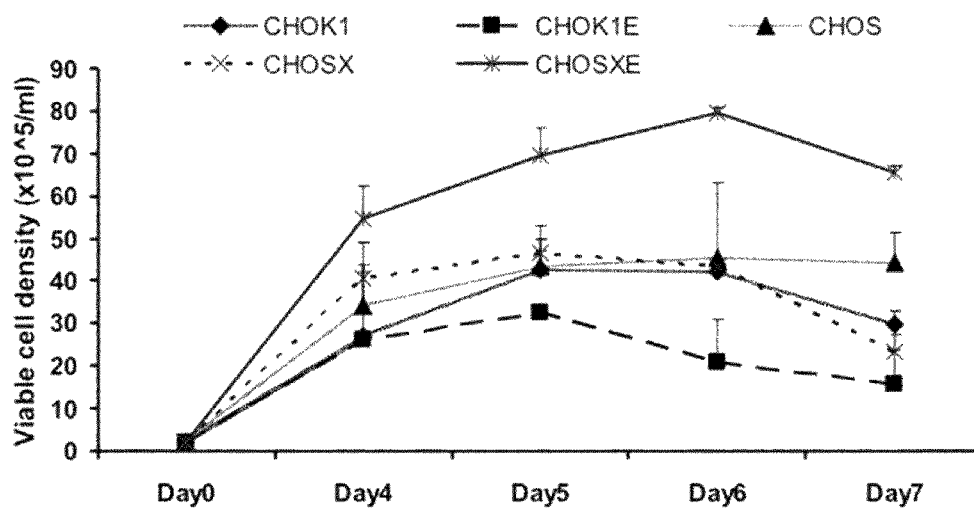


Figure 8

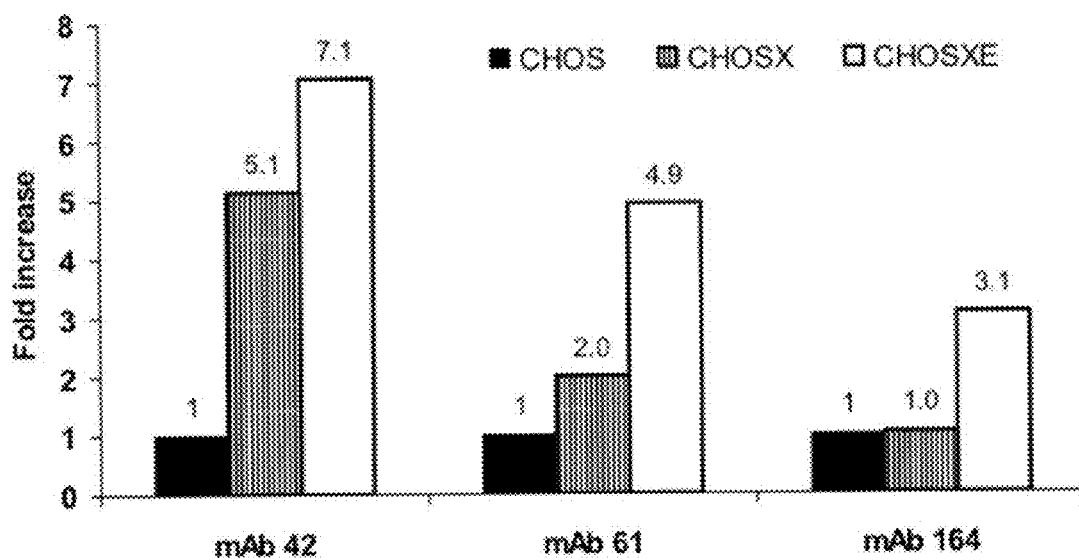


Figure 9

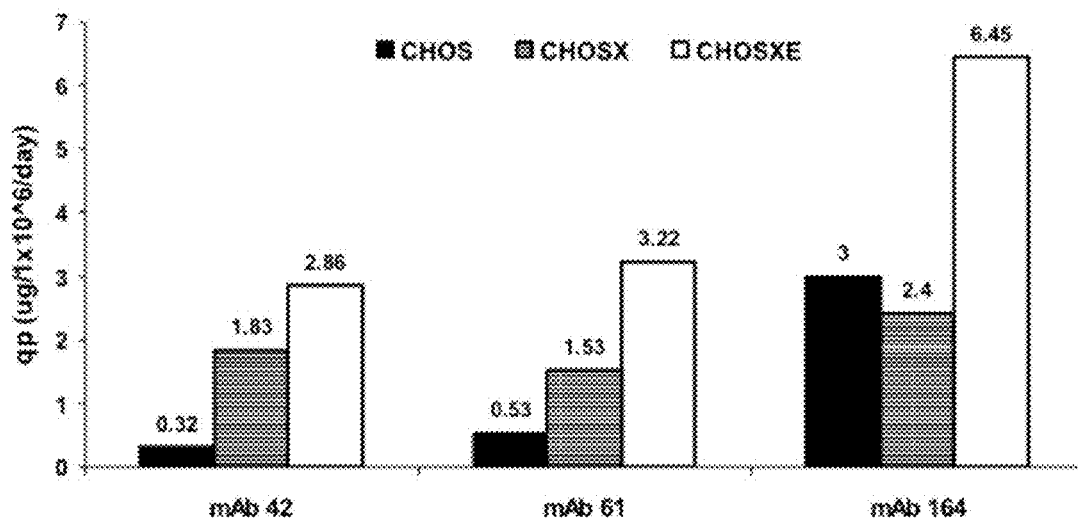


Figure 10

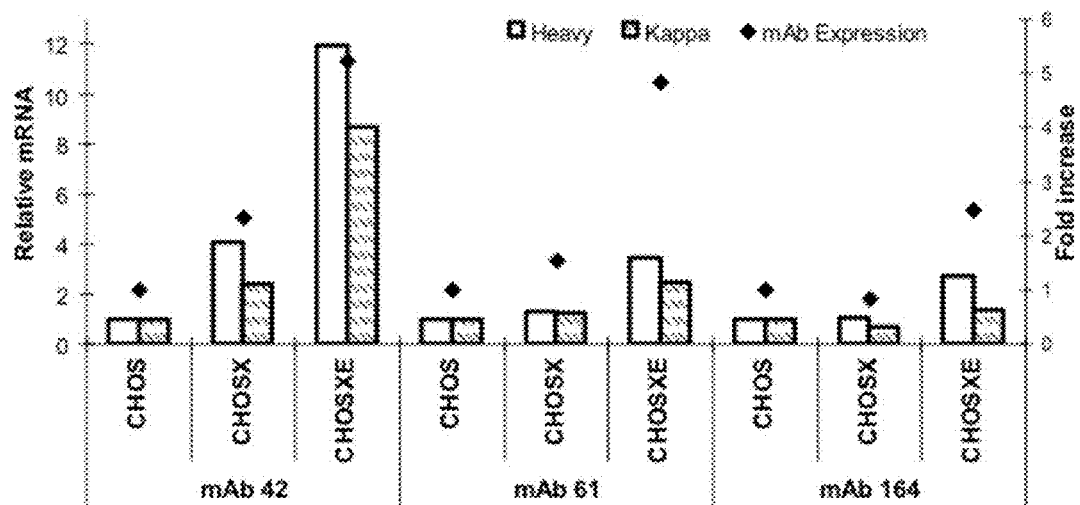


Figure 11

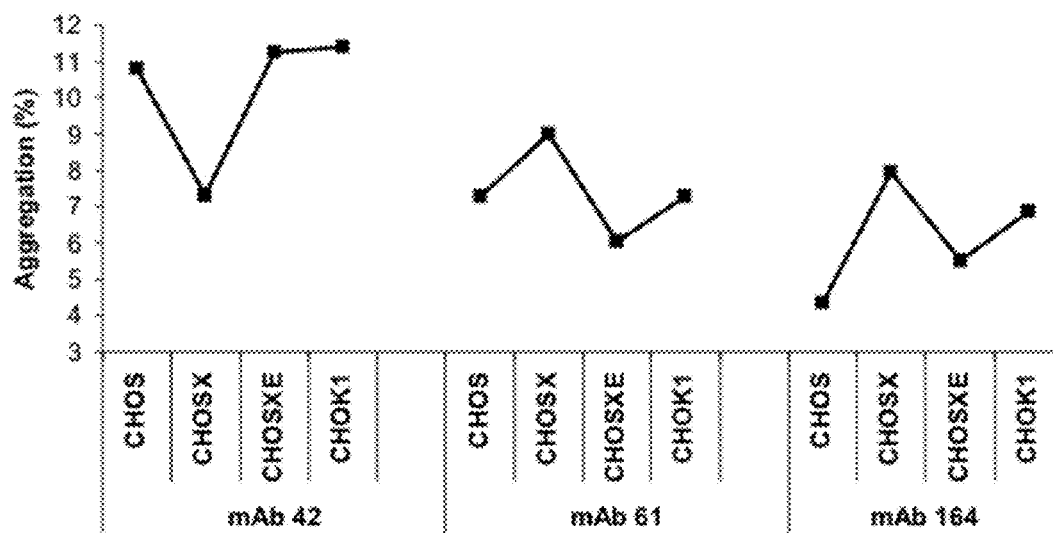


Figure 12a

mAb 42

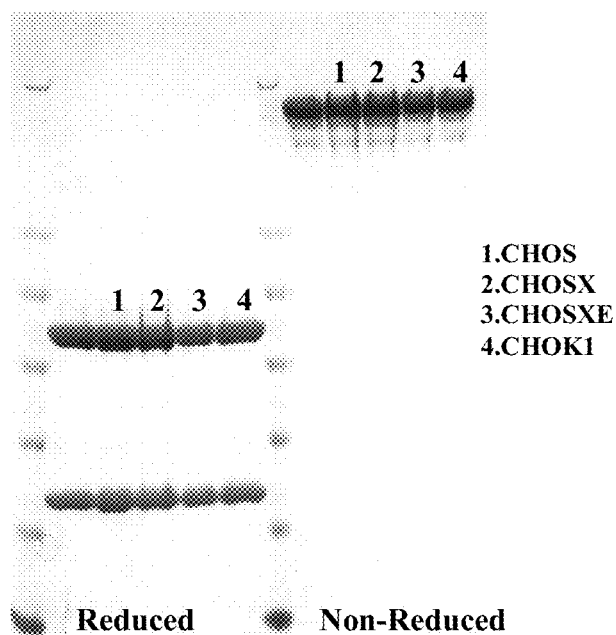


Figure 12b

mAb 61

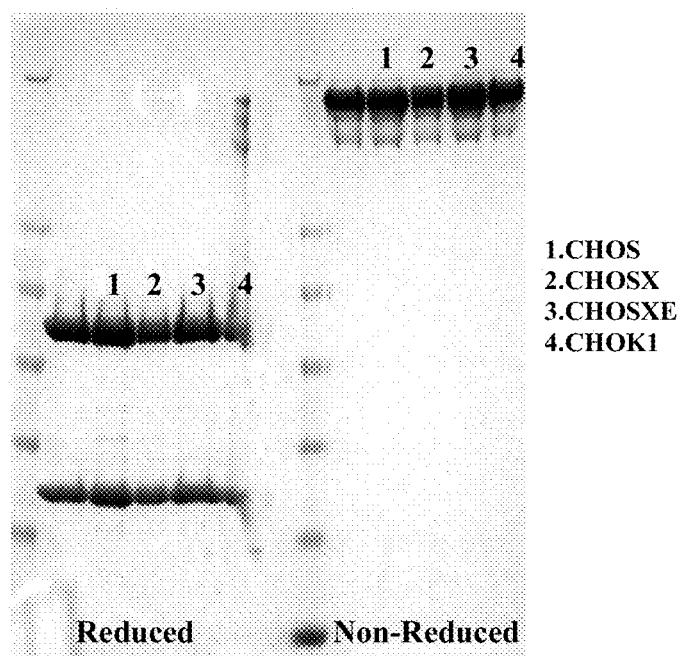


Figure 12c

mAb 164

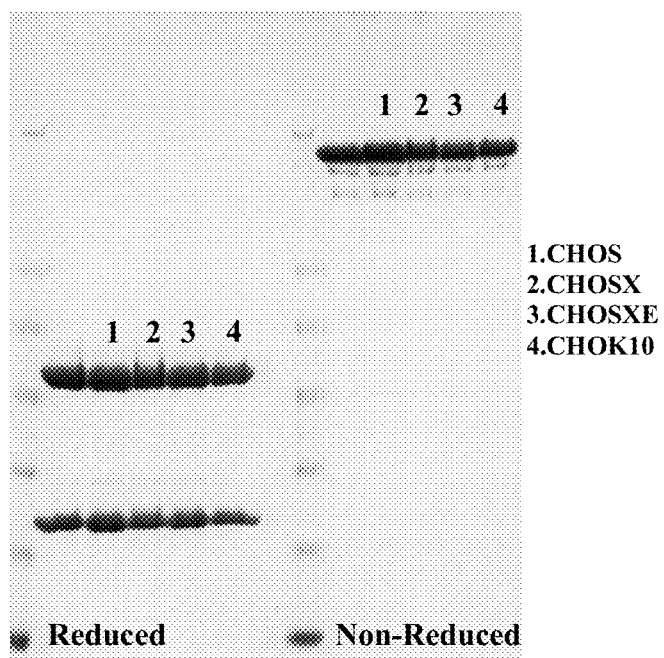


Figure 13

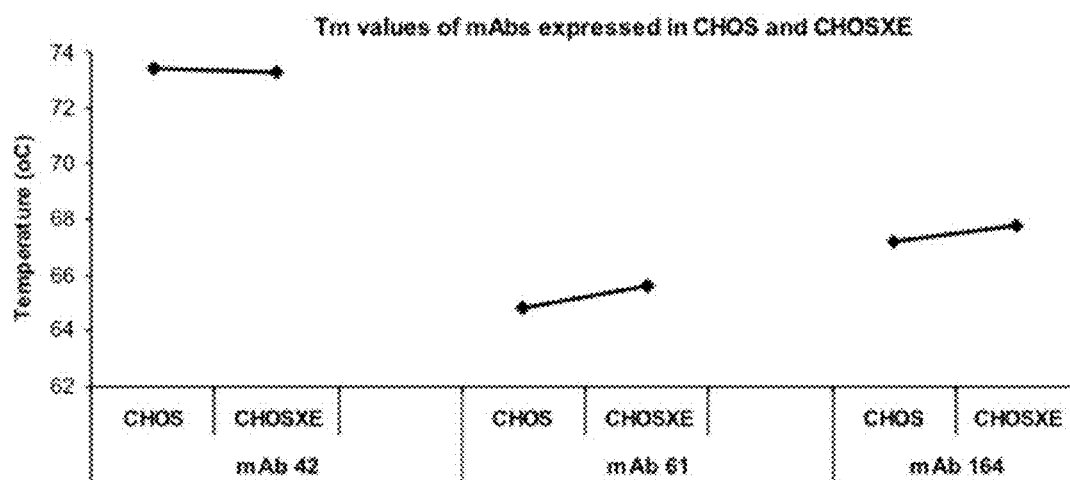


Figure 14

		mAb 42		mAb 61		mAb 164		Average	
		CHOS	CHOS XE	CHOS	CHOS XE	CHOS	CHOS XE	CHOS	CHOS XE
	G0F	47	53	37	48	49	50	44	50
	G1F	21	10	17	11	24	18	21	13
	G2F	2	0	1	0	1	1	2	1
	M3N3F	1	1	1	2	1	2	1	2
	M3N2F	1	0	0	0	0	0	0	0
	G0	7	22	11	26	10	16	9	21
	G1	9	5	17	5	6	8	11	6
	G2	0	1	6	1	2	1	3	1
	M3N3	0	3	1	1	0	2	0	2
	M3N2	1	0	1	0	0	0	0	0
	M5	9	5	8	5	6	2	8	4
	M6	2	1	0	0	0	0	1	0
	M7	0	0	0	0	0	0	0	0
	M8	0	0	0	0	0	0	0	0
	M9	0	0	0	0	0	0	0	0
	Fucosylated	72	65	55	62	76	71	68	66
	Non-fucosylated	17	30	36	33	18	27	24	30
	High mannose	11	5	9	5	6	2	9	4

Figure 15

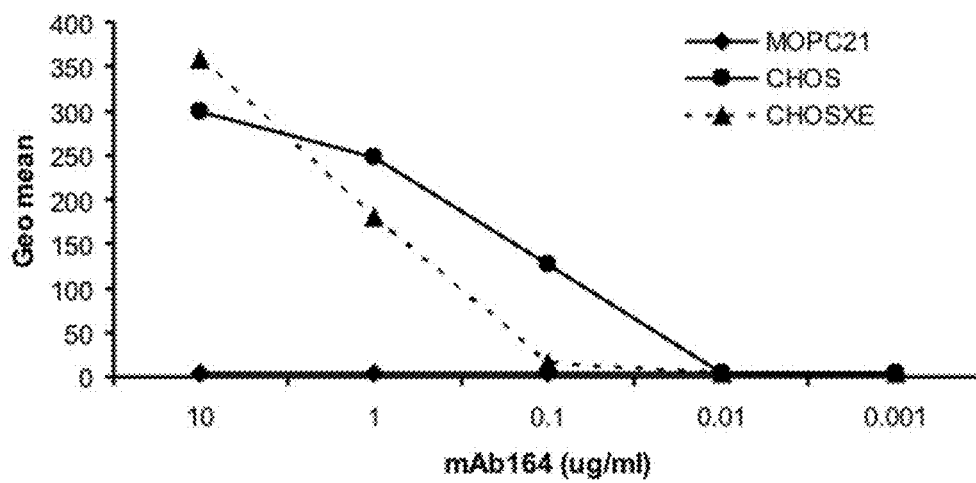


Figure 16

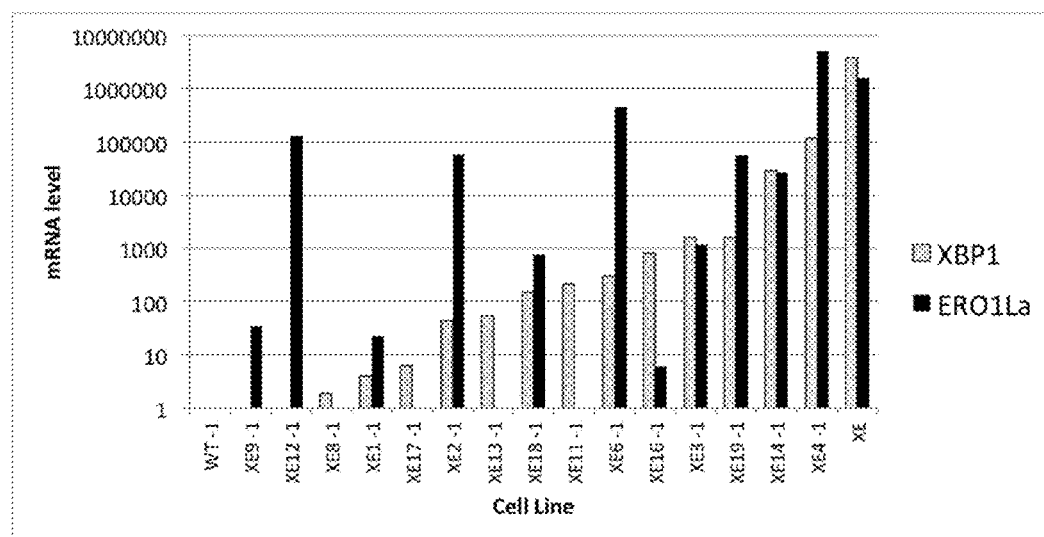


Figure 17

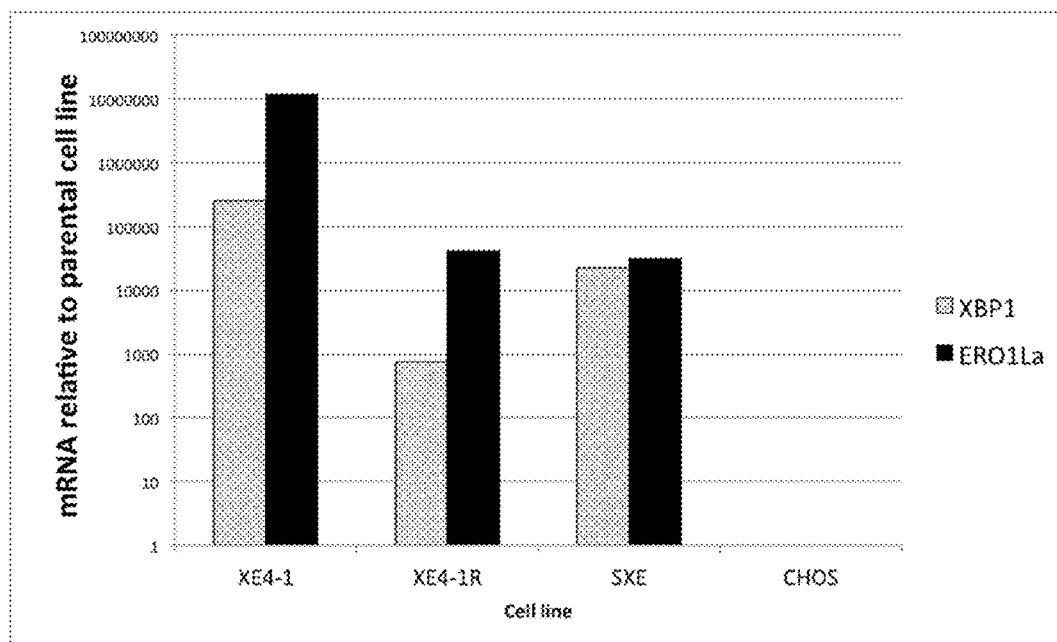


Figure 18

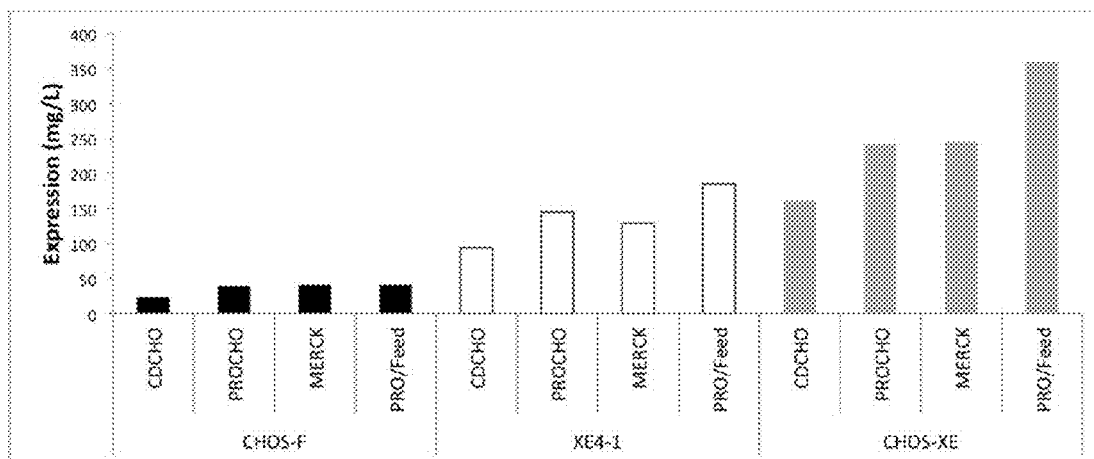


Figure 19

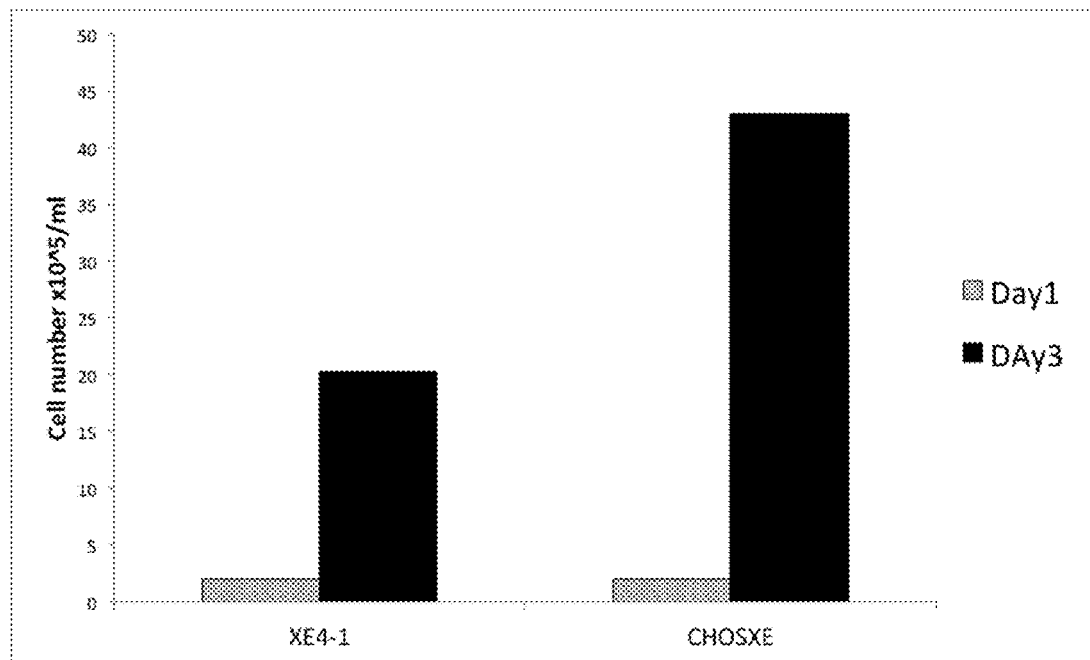


Figure 20

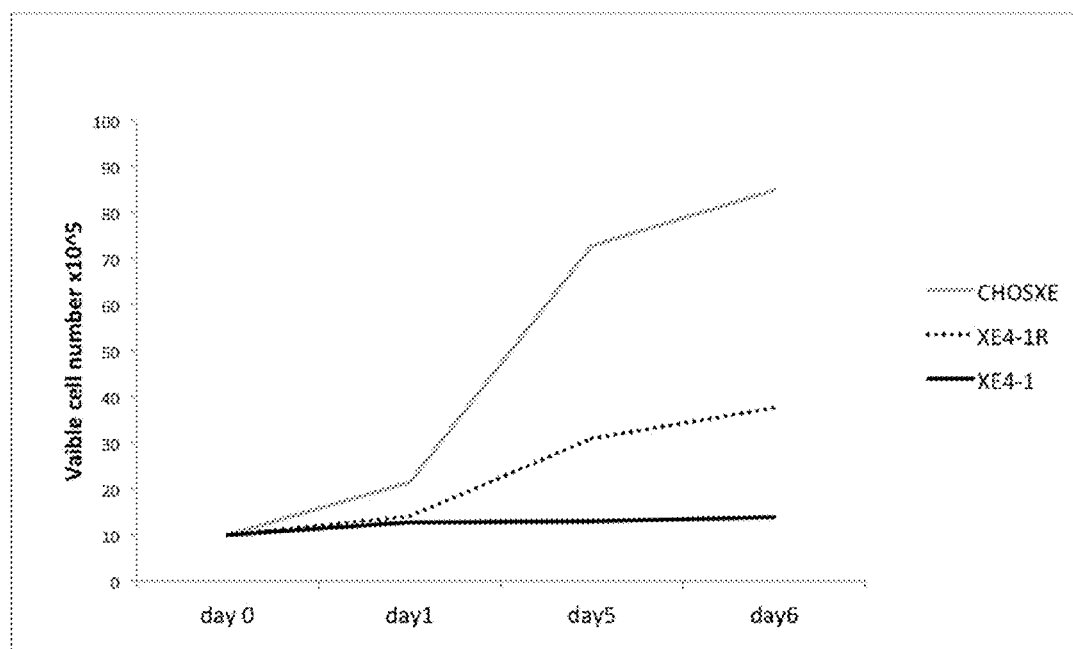
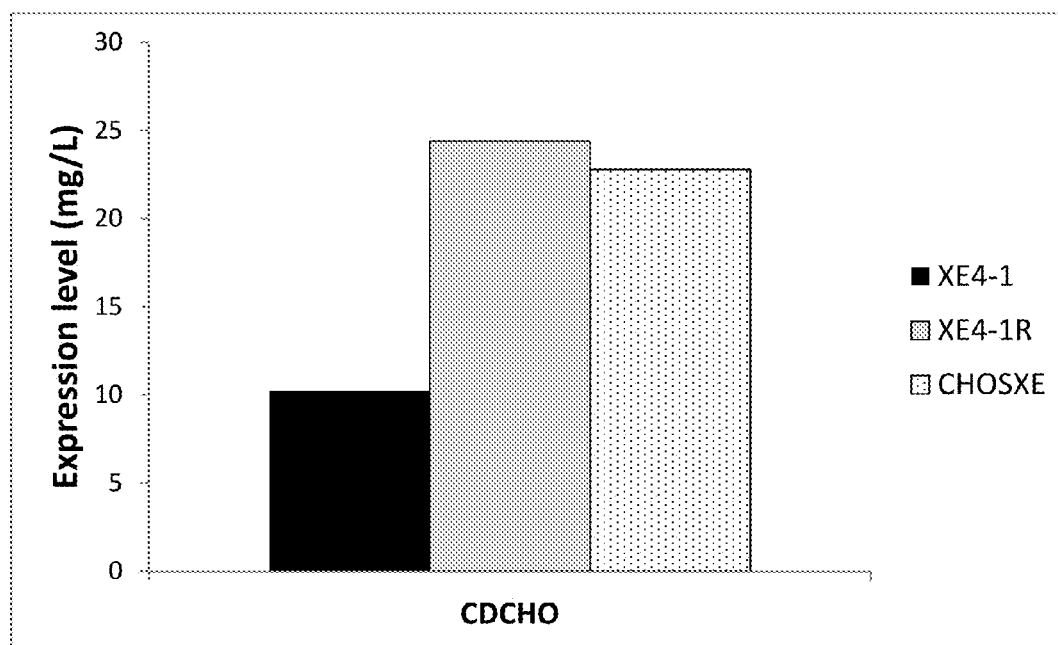


Figure 21



METHOD FOR PRODUCING PROTEIN**CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a continuation-in-part of U.S. patent application Ser. No. 13/148,740 filed on Nov. 30, 2011, which is a U.S. National Phase of International Application No. PCT/GB2010/000234 filed Feb. 10, 2010, which claims priority from GB Patent Application No. 0902180.9 filed Feb. 10, 2009. The entire disclosure contents of these applications are hereby incorporated by reference into the present application.

BACKGROUND OF THE INVENTION**1. Field of the Invention**

The present invention relates to a recombinant host cell capable of improved production of a protein of interest. The invention also relates to a method for improving the capability of a cell to produce a protein of interest and a method for producing a protein of interest in such a cell.

2. Description of Related Art

One of the most important functions of endoplasmic reticulum (ER)-Golgi system is to facilitate post-translational modifications including protein folding. The correct folding of newly-translated proteins is essential for secreted extracellular proteins and membrane proteins to ensure that they can function correctly. Protein folding allows a polypeptide to fold into its characteristic and functional three-dimensional structure. Protein folding involves the formation of both non-covalent and covalent bonds. Generally in eukaryotic cells, protein folding is mediated in the rough ER by ER chaperone proteins.

Protein disulphide isomerase (PDI) is a chaperone protein which catalyzes the formation and isomerisation of disulphide bonds between two cysteine residues in polypeptides. The formation of disulphide bonds is a redox reaction. Proteins are oxidized by PDI when it is in an oxidized state, which produces a disulphide bond in the protein and renders PDI in a reduced state comprising free sulfhydryl groups.

ER oxidoreductin 1 protein (Ero1) is an essential component of oxidative folding machinery. Ero1 is a flavin adenine dinucleotide (FAD) dependent enzyme which facilitates disulphide bond formation in immunoglobulin subunits by selectively oxidizing PDI (Mezghrani et al., 2001, EMBO Journal, 20(22), 6288-6296). In humans two isoforms have been identified as human Ero1-L α and Ero1-L β .

U.S. Pat. No. 6,361,964 discloses expression systems that make use of Ero1 to enhance disulphide bond formation and thereby to increase the yield of properly folded recombinant proteins.

The unfolded protein response (UPR) allows cells to respond to an increased demand on the protein folding capacity of the ER by coordinating the down regulation of protein synthesis with the increased expression of various proteins including ER resident chaperone proteins and folding enzymes which enable protein folding (Gunn et al., 2004, Molecular Immunology, 41, 919-9927). The UPR may also cause enlargement of the ER in order to provide increased capacity for protein folding.

X Box Binding Protein 1 (XBP1) is known to be a key regulator of the unfolded protein response. XBP1 is a transcription factor which is involved in B cell differentiation, ER expansion by stimulating activity of enzymes involved in lipid biosynthesis and regulates gene encoding ER resident chaperone proteins involved in the UPR (Lee et al., 2003,

Molecular and Cellular Biology, 23(21), 7448-7459). XBP1 is believed to cause enlargement of the ER by up-regulating or activating enzymes including choline cytidyltransferase (CCT). CCT is the rate-limiting enzyme in the CDP-choline pathway for the production of phosphatidylcholine (PtdCho), which is the primary phospholipid of the ER membrane.

The use of XBP1 in methods for protein production wherein a protein of interest is co-expressed with XBP1 are described in WO 2004/111194, WO 2006/028889 and US2005/0250182.

Accordingly, methods to improve the yield of protein expression systems using a component of the UPR pathway are known. However, there is still a need to provide improved methods for increasing the yield of proteins in expression systems.

SUMMARY OF THE INVENTION

The present invention provides a recombinant host cell capable of improved yield of a protein of interest. The present inventors have demonstrated that an increase of both Ero1 and XBP1 in a cell provides improved means for providing a protein of interest.

Hence, the present invention provides a recombinant host cell, wherein the cell is modified to increase expression levels of Ero1 and XBP1 relative to the expression levels of Ero1 and XBP1 in an unmodified cell.

The present invention also provides a cell comprising an agent suitable for increasing the levels of Ero1 and XBP1 in the cell. Any suitable agent may be used for increasing the levels of Ero1 and XBP1. Typically the agent is one or more exogenous polynucleotide sequences encoding Ero1 and XBP1. Accordingly, the present invention provides a cell comprising one or more exogenous polynucleotide sequences encoding Ero1 and XBP1. A further typical agent may be capable of modulating endogenous polynucleotides encoding Ero1 and XBP1 in order to increase Ero1 and XBP1 expression. Accordingly, the present invention also provides a cell comprising an agent capable of increasing endogenous Ero1 and XBP1 expression.

The cell provided by the present invention is advantageous because in one embodiment it increases the yield of a protein of interest. In a further embodiment the present method increases the cell's capacity to perform post-translational modifications. The present method may initiate or up-regulate the UPR pathway. Further, the protein of interest expressed by the cell according to the present invention may have substantially the same properties as when the protein is expressed by an unmodified cell.

The present invention also provides a method comprising modifying a cell to thereby increase the capability of the cell to increase the expression levels of Ero1 and XBP1 relative to the expression levels of Ero1 and XBP1 in an unmodified cell.

In one aspect of the present invention the method is for expressing a protein of interest. The present inventors have found that the over expression of Ero1 and XBP1 significantly improves the cell's capability to express a protein of interest resulting in higher yields of the protein of interest.

The present invention also provides an isolated polynucleotide comprising a polynucleotide sequence encoding Ero1 or a variant thereof which substantially retains the function of Ero1; and a polynucleotide sequence encoding XBP1 or a variant thereof which substantially retains the function of XBP1.

In some aspects, the cell provided by the invention is from a CHOSXE cell line as deposited at the European Collection of Cell Cultures (ECACC) having the Accession number: 10021001.

In some aspects, the cell provided by the invention is from an XE4-1R cell line as deposited at the European Collection of Cell Cultures (ECACC) having the Accession number: 14032501.

In some aspects, the cell provided by the invention further comprises a polynucleotide sequence encoding a protein of interest, wherein the protein of interest is exogenous.

In some aspects, the polynucleotide sequence encoding the protein of interest is within an expression cassette that does not comprise a nucleic acid sequence encoding Endoplasmic Reticulum Oxidoreductin 1 (Ero1), a nucleic acid sequence encoding X-box binding protein 1 (XBP1), or a combination thereof.

In some aspects, the protein of interest is an antibody or an antigen binding fragment thereof.

In some aspects, the cell provided by the invention is further modified to increase the expression levels of a further component of the unfolded protein response (UPR) pathway relative to the expression levels of the further component of the UPR pathway in an unmodified cell not having increased expression of the further component of the UPR pathway.

In some aspects, the cell provided by the invention further comprises an exogenous polynucleotide sequence encoding the component of the unfolded protein response (UPR) pathway.

The invention further provides methods of producing a recombinant protein of interest comprising expressing the recombinant protein of interest in a cell provided by the invention.

In some aspects, the cell provided by the invention is for use as a medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following drawings specific embodiments of the present invention are described by way of example only, in which:

FIG. 1a shows the amino acid sequence of the spliced form of human XBP1 (hXBP1s) (SEQ ID NO:1). The * denotes a stop codon.

FIG. 1b shows the nucleic acid sequence of the spliced form of human XBP1 (hXBP1s) (SEQ ID NO:2).

FIG. 2a shows the amino acid sequence of human Ero1 α (hEro1 α) (SEQ ID NO:3).

FIG. 2b shows the nucleic acid sequence of human Ero1 α (hEro1 α) (SEQ ID NO:4).

FIG. 3a shows hEro1 α mRNA expression and FIG. 3b shows hXBPs mRNA expression in CHOSXE cell clones (17, 25, 77, 18, 78, 70, 103, 85, 87, 67, 176, 36, 173, 88, 30, 76, 89, 86, 94 and 101), CHOSX and CHOK1E relative to CHOS after a first picking step of CHOSXE cell clones.

FIG. 4 shows hEro1 α mRNA and hXBPs mRNA expression after pick 2 of CHOSXE cell clones in CHOSXE cell clones (23, 64, 19, 22, 11, 69, 53, 72, 16, 62, 13, 63, 25, 45, 10, 55, 20, 71, 52, 49 and 57), CHOSX and CHOK1E relative to CHOS after a second picking step of CHOSXE cell clones.

FIG. 5 shows the relative increase the expression of antibodies 632 and 497 in cell clones from a CHOS cell line transfected with hEro1 α and hXBP1s (CHOSXE) and a CHOS cell line transfected with hXBP1s (CHOSX) compared to expression of antibodies 632 and 497 in a CHOS cell line.

FIG. 6 shows the relative increase the expression of antibodies 146 and 240 from cell clones from a CHOS cell line transfected with hEro1 α and hXBP1s (CHOSXE), a CHOS cell line transfected with hXBP1s (CHOSX) and a CHOK1 cell line transfected with hEro1 α (CHOK1E) compared to expression of antibodies 146 and 240 from a CHOS cell line.

FIG. 7 shows a growth curve of cell lines CHOS, CHOSX, CHOSXE, CHOK1 and CHOK1E.

FIG. 8 shows the relative increase in expression of antibodies 42, 61 and 164 in CHOSXE and CHOSX compared to the expression of 42, 61 and 164 in CHOS cells.

FIG. 9 shows the specific productivity of CHOS, CHOSX and CHOSXE cell lines between day 4 and day 7 for antibodies 42, 61 and 164.

FIG. 10 shows the relative heavy chain and kappa light chain mRNA expression for antibodies 42, 61 and 164 expressed in CHOSX and CHOSXE cell lines compared to CHOS cell line.

FIG. 11 shows the % aggregation of antibodies 42, 61 and 164 expressed in CHOK1, CHOS, CHOSX and CHOSXE.

FIG. 12a shows an SDS PAGE of antibody 42 expressed in CHOS, CHOSX CHOSXE and CHOK1; FIG. 12b shows an SDS PAGE of antibody 61 expressed in CHOS, CHOSX CHOSXE and CHOK1; and FIG. 12c shows an SDS PAGE of antibody 164 expressed in CHOS, CHOSX CHOSXE and CHOK1.

FIG. 13 shows the results from a thermofluor assay of antibodies 42, 61 and 164 expressed in CHOS and CHOSXE.

FIG. 14 shows the glycosylation profiles of antibodies 42, 61 and 164 expressed in CHOS and CHOSXE.

FIG. 15 shows the results from an antigen binding assay of 164 expressed in CHOS and CHOSXE.

FIG. 16 shows hXBP1 and hEro1 α levels of cell lines generated from clones of CHOS cells transfected with a vector comprising hXBP1(s) and hEro1 α cDNA.

FIG. 17 shows hXBP1(s) and hEro1 α levels in the XE4-1, XE4-1R, CHOSXE, and CHOS cell lines.

FIG. 18 shows the relative expression of mAb X in CHOS-F, XE4-1, and CHOSXE cells.

FIG. 19 shows that CHOSXE cells divide at a faster rate than XE4-1 cells.

FIG. 20 shows the numbers of CHOSXE, XE4-1R, and XE4-1 cells following transfection with antibody DNA at day 0.

FIG. 21 shows antibody yields of CHOSXE, XE4-1R, and XE4-1 cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a recombinant host cell, which has improved capacity to produce a protein of interest due to modification of the cell to increase Ero1 and XBP1 expression. The present inventors have found that an increase of Ero1 and XBP1 in a host cell significantly improves protein yield compared to an unmodified cell. Preferably the cell according to the present invention has a specific productivity of a protein of interest of 80% or more, 85% or more, 90% or more, 100% or more, 150% or more, 200% or more, 300% or more, 400% or more, 500% or more, 600% or more, 700% or more or 800% or more relative to control cells which have not been modified to increase Ero1 and XBP1. More preferably the cell according to the present invention has a specific productivity of a protein of interest of from 100% to 1000%, more preferably 200% to 1000%, still more preferably 500% to 1000% relative to control cells, which have not been modified to increase Ero1 and XBP1.

Moreover, the present inventors have found that the cell according to the present invention has improved protein yield compared to a cell over expressing either Ero1 or XBP1 with the protein of interest.

In one embodiment the cell according to the present invention has a specific productivity of a protein of interest greater than the combined cellular productivity of a protein of interest from a cell modified to increase the expression of Ero1 but not XBP1 and a cell modified to increase XBP1 but not Ero1. Preferably the cell according to the present invention has a specific productivity of a protein of interest of 5% or more, 6% or more, 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more 80% or more, 90% or more, 100% or more, 110% or more, 120% or more, 130% or more, 140% or more, 150% or more, more preferably 5% to 150%, 6% to 130%, 10% to 130% or 50% to 130% relative to the combined cellular productivity of a protein of interest from a cell modified to increase the expression of Ero1 but not XBP1 and a cell modified to increase XBP1 but not Ero1.

Preferably the cell according to the present invention has a specific productivity of a protein of interest of 2 or more $\mu\text{g}/1 \times 10^6$ cells/day, preferably 3 or more $\mu\text{g}/1 \times 10^6$ cells/day, more preferably more than 3 $\mu\text{g}/1 \times 10^6$ cells/day, still more preferably 3.2 or more $\mu\text{g}/1 \times 10^6$ cells/day, more preferably 3.2 to 6.5 $\mu\text{g}/1 \times 10^6$ cells/day.

The skilled person would easily be able to test a candidate cell clone to see if it has the desired yield of a protein of interest using methods well known in the art, such as ELISA.

In a further embodiment the co-expression of both Ero1 and XBP1 provides improved yield of correctly folded proteins from the cell relative to unmodified cells. The skilled person would easily be able to test secreted protein to see if the protein is correctly folded using methods well known in the art, such as protein G HPLC, circular dichroism, NMR and X-Ray crystallography.

Without wishing to be bound by theory it is believed that the improved protein expression is due to the increased capacity of the cell to fold proteins. Specifically, increase of Ero1 expression increases the cell's capacity to form disulphide bonds in protein in the ER. In addition, the increase of XBP1 expression causes up-regulation of ER chaperone protein expression and may enlarge the size of the ER. The cell provided by the present invention may also provide an increased yield of protein relative to unmodified cells due to the increased capacity of the cell to fold all proteins expressed by the cell, which thereby increases all cellular pathways including transcription, translation, protein folding and secretory pathways. Further, due to the increase of Ero1 and XBP1 increasing the cell's capacity to process newly translated proteins, any feedback control of mRNA transcription and/or protein translation may cause an increase in protein transcription and/or translation.

In a further embodiment the co-expression of both Ero1 and XBP1 in the cell of the invention provides improved cell growth and/or stability and/or reproduction relative to unmodified cells. Preferably the cells provided by the present invention have increased cell density after the same period of culture of 30% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, 100% or more, 130% or more, 150% or more, 170% or more, or 190% or more compared to the cell density of a culture of control cells which have not been modified to up-increase Ero1 and XBP1.

The skilled person would easily be able to select cells having improved cell growth and/or stability and/or reproduction by, for example, measuring the cell density and/or cell viability of a cell culture after a fixed culture period.

Suitable methods known the art include the CEDEX (Innovatis) automated cell counting system based on the well-established Trypan Blue exclusion method for determining cell viability. Sample handling, staining, cell counting and graphical analysis of the results are performed automatically by the CEDEX system.

Without wishing to be bound by theory it is believed that the improved cell growth and/or stability are due to the increased capacity of the cell to process all proteins which require post-translational modifications in the ER, which thereby increases cellular pathways. Accordingly, the production of correctly folded proteins necessary for the cell's growth and reproduction may be increased thereby improving the cellular pathways regulating growth and/or stability and/or reproduction.

These findings are unexpected, particularly because XBP1 and Ero1 are not linked in a UPR pathway. The UPR is extremely complex and involves numerous different pathways. Whilst co-expression of either XBP1 or Ero1 with additional components or modulators of the same UPR pathway may be expected to improve protein expression, it is surprising that co-expression of two unconnected proteins would provide a significantly improved protein expression system.

The present invention will now be described in more detail. The terms "protein" and "polypeptide" are used interchangeably herein, unless the context indicates otherwise. "Peptide" is intended to refer to 10 or less amino acids.

The terms "polynucleotide" includes a gene, DNA, cDNA, RNA, mRNA etc unless the context indicates otherwise.

As used herein, the term "comprising" in context of the present specification should be interpreted as "including".

In one aspect of the present invention there is a provided a recombinant host cell modified to increase/up-regulate/over-express the expression levels of Ero1 and XBP1 relative to the expression levels of Ero1 and XBP1 in an unmodified cell.

The unmodified cell or control cell in the context of the present invention means a cell of the same type as the host cell wherein the cell has not been modified to increase/up-regulate/over-express the expression levels of Ero1 and XBP1, for example the unmodified cell may be derived from a population of host cells before modification to increase/up-regulate/over-express the expression levels of Ero1 and XBP1.

In one embodiment the cell comprises an exogenous recombinant polynucleotide sequence encoding Ero1 or a variant thereof which substantially retains the function of Ero1; and an exogenous recombinant polynucleotide encoding XBP1 or a variant thereof which substantially retains the function of XBP1. In this embodiment the cell may comprise a polynucleotide encoding both Ero1 and XBP1 and/or the cell may comprise separate polynucleotides encoding Ero1 and XBP1.

As used herein, "the polynucleotide," "the polynucleotide encoding Ero1," "the polynucleotide encoding XBP1," and "the polynucleotide encoding Ero1 and XBP1" are intended to refer to all aspects and embodiments of the present invention wherein Ero1 and XBP1 may be encoded by the same and/or separate polynucleotides, unless stated otherwise.

Accordingly, the cell according to the present invention may comprise an exogenous Ero1 and XBP1 polynucleotide comprising both a polynucleotide sequence encoding Ero1 and a polynucleotide sequence encoding XBP1. The cell according to the present invention may also comprise such a polynucleotide in the form of an expression cassette or a vector.

Alternatively or additionally, the cell according to the present invention comprises an exogenous Ero1 polynucle-

otide comprising the polynucleotide sequence encoding Ero1 and a separate exogenous XBP1 polynucleotide comprising the polynucleotide sequence encoding XBP1. Accordingly, the cell according to the present invention may comprise an Ero1 expression cassette or vector comprising the Ero1 polynucleotide and a separate XBP1 expression cassette or vector comprising the XBP1 polynucleotide.

In some embodiments, human XBP1 is first cloned into a vector and transfected into CHOS cells to produce CHOSX cells. In further embodiments, CHOSX cells are subsequently transfected with a separate vector comprising human Ero1 to produce CHOSXE cells.

In some embodiments, an expression cassette or vector comprises an Ero1 polynucleotide and an XBP1 polynucleotide. In some embodiments, Ero1 and XBP1 are housed in separate cassettes in one mammalian expression vector. In some embodiments, human XBP1 cDNA is cloned into a multiple cloning site located between a CMV promoter and SV40PolyA and human Ero1 cDNA is cloned into a multiple cloning site between a second CMB promoter and SV40PolyA of a mammalian expression vector. In some embodiments, the expression vector comprising Ero1 and XBP1 is transfected into CHOS cells by electroporation. In some embodiments, the transformed CHOS cells are XE4-1 cells or XE4-1R cells.

In a further embodiment the increased Ero1 and XBP1 expression is from modulation of endogenous polynucleotides encoding Ero1 and XBP1 in the cell according to the present invention. In this embodiment the cell is modified to increase transcription and translation of the endogenous genes of Ero1 and XBP1.

The cell according to the present invention may further comprise a polynucleotide sequence encoding a protein of interest. The polynucleotide sequence encoding the protein of interest may be exogenous or endogenous. The polynucleotide sequence encoding the protein of interest may be integrated into the host's chromosome or may be non-integrated in an episome.

In the embodiment wherein the polynucleotide sequence encoding the protein of interest is exogenous and the cell comprises one or more exogenous polynucleotides encoding Ero1 and XBP1, the polynucleotide sequence encoding the protein of interest may be part of one or more of the Ero1 and/or XBP1 encoding exogenous polynucleotides. Accordingly, the polynucleotide sequence encoding a protein of interest may be in the same polynucleotide sequence as both XBP1 and Ero1 e.g. the same expression cassette or vector. Alternatively or additionally the polynucleotide sequence encoding a protein of interest may be in the same polynucleotide sequence as either the separate Ero1 polynucleotide sequence and/or the separate XBP1 polynucleotide sequence e.g. the same expression cassette or vector. Alternatively or additionally, the cell may further comprise a separate polynucleotide, an expression cassette or a vector comprising the polynucleotide sequence encoding the protein of interest.

Suitable cells for employing in the invention include eukaryotic cells, for example plant cells, insect cells, yeast cells, animal cells such as mammalian cells, in particular CHO cells, myeloma cells, viro cells, MRC5 Cells, HEK cells, NSO cells, SP2 cells and the like.

CHO cells have been found to be particularly useful for mammalian expression because the proteins expressed in CHO cells have glycoforms that are generally compatible and bioactive in humans. Accordingly, in one aspect the invention employs a mammalian cell such as a CHO cell, for example a CHOS cell (Invitrogen Cat. No. 11619-012, Deaven, L. L. et al, 1973, Chromasoma 41, 129, D'Anna, J. A. et al, 1996,

Methods in Cell Science 18, 115, D'Anna, J. A. et al, 1997, Radiation Research 148, 260) or CHOK1 cell (Puck, T. et al. 1967, Genetics of Somatic Mammalian Cells IV Properties of Chinese Hamster Cell Mutants With Respect To The Requirement For Proline; Genetics; 55; 513-524; March 1967), or a derivative therefrom.

In some embodiments, the cell provided by the present invention is the modified CHOS cell strain Depositor Deposit Name: CHOSXE (Depositor Identification in Full: CHOS.Xbp1.Ero1a), which was deposited on 10 Feb. 2010 by UCB Celltech, the UK branch of UCB Pharma S.A., at the European Collection of Cell Cultures (ECACC), HPA, United Kingdom, under HPA Culture Collections Reference Number: Q8515 and Accession number: 10021001 in accordance with The Budapest Treaty, and all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of this patent.

In some embodiments, the cell provided by the present invention is the modified CHOS cell strain Depositor Deposit Name: XE4-1R, which was deposited on 25 Mar. 2014 by UCB Celltech, the UK branch of UCB Pharma S.A., at the European Collection of Cell Cultures (ECACC), HPA, United Kingdom, under Accession number: 14032501 in accordance with The Budapest Treaty.

As used herein, "Ero1" means a polypeptide having the activity of facilitating disulphide bond formation by oxidizing PDI. Ero1 polypeptide is formerly known as Sec81.

Any suitable form of Ero1 from any suitable source may be used in the present invention including Ero1 α and Ero1 β . Typically human Ero1-L α and/or Ero1-L β may be used in the present invention.

In one embodiment, the polynucleotide encoding Ero1 used in the present invention encodes a variant polypeptide of the endogenous form of Ero1, which substantially retains the activity of oxidizing PDI.

A variant polypeptide in the context of the present specification is intended to refer to a sequence that has one or more amino acid substitutions, deletions or insertions compared to the endogenous polypeptide but retains all or substantially all of the function such as 80% or more, in particular 90% or more, for example 95% or more or 100% of the activity of the endogenous polypeptide.

The polynucleotide sequence encoding Ero1 used in the present invention preferably encodes an Ero1 α polypeptide sequence comprising the polypeptide sequence shown in SEQ ID NO: 3. Amino acids 1 to 23 of SEQ ID NO:3 encode a signal peptide. Accordingly the polynucleotide sequence encoding Ero1 used in the present invention may or may not comprise the signal peptide and therefore may comprise amino acids 1 to 468 or amino acids 24 to 468. It will be appreciated that one or more amino acid substitutions, insertions or deletions may be made to SEQ ID NO: 3 without significantly altering the activity of Ero1. The effect of any amino acid substitutions, insertions or deletions can be readily tested by one skilled in the art by any suitable method. For example, an anti-Ero1 antibody may be used to determine Ero1 presence and the redox environment of the cell may be tested to determine Ero1 activity. Accordingly, in one embodiment, the polynucleotide sequence encoding Ero1 of the present invention encodes a polypeptide sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 3.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned

sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to: phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains); lysine, arginine and histidine (amino acids having basic side chains); aspartate and glutamate (amino acids having acidic side chains); asparagine and glutamine (amino acids having amide side chains); and cysteine and methionine (amino acids having sulphur-containing side chains).

Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, N.J., 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

It will be appreciated that any suitable polynucleotide sequence encoding an Ero1 polypeptide may be used. In one embodiment the polynucleotide sequence encoding Ero1 comprising the polynucleotide sequence shown in SEQ ID NO:4. The polynucleotide sequence shown in SEQ ID NO:4 may have one or more nucleotide substitutions, insertions or deletions without significantly altering the activity of Ero1. Accordingly, in one embodiment, the polynucleotide sequence encoding Ero1 comprises a sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 4. In a further embodiment, the invention encompasses the use of polynucleotides that are complementary to, antisense to or hybridize under stringent conditions to the polynucleotide described herein.

The Ero1 employed in the present invention may be derived from any suitable source, for example human, mouse, hamster or yeast.

As used herein, "XBP1" means a polypeptide having transcription activation activity for one or more UPR genes including ER chaperone proteins.

Any suitable form of XBP1 from any suitable source may be used in the present invention.

Two isoforms of XBP-1 exist resulting from alternative splicing events. The XBP-1 gene is transcribed into a mRNA which encodes an unspliced version of XBP-1 and a shorter spliced mRNA is also generated which lacks exons. XBP-1 mRNA is spliced in response to ER stress, such as the accumulation of unfolded protein in the ER. It has been shown that only the spliced form of XBP-1 can efficiently activate the UPR pathway (Yoshida et al., 2001, Cell, 107, 881-891). Accordingly, the polynucleotide according to the present invention preferably encodes the spliced form of XBP1.

In one embodiment, the polynucleotide encoding XBP1 used in the present invention encodes a variant polypeptide of the endogenous form of XBP1 which substantially retains the transactivator activity of XBP1.

The polynucleotide sequence encoding XBP1 used in the present invention preferably encodes a polypeptide sequence comprising the polypeptide sequence shown in SEQ ID NO: 1. It will be appreciated that one or more amino acid substitutions, insertions or deletions may be made to SEQ ID NO: 1 without significantly altering the activity of XBP1. The effect of any amino acid substitutions, insertions or deletions

can be readily tested by one skilled in the art. For example, a BODIPY staining method may be used to measure the size of the ER because XBP1 activity increases the size of the ER. The activity of choline cytidyltransferase alpha (CCTalpha) may also be used to determine XBP1 activity because the activity of CCT alpha is increased by XBP1 over-expression.

In one embodiment, the polynucleotide sequence encoding XBP1 of the present invention encodes a polypeptide sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 1.

It will be appreciated that any suitable polynucleotide sequence encoding an XBP1 polypeptide may be used. In one embodiment the polynucleotide sequence encoding XBP1 comprises the polynucleotide sequence shown in SEQ ID NO:2, wherein SEQ ID NO:2 is the spliced form of XBP1. The polynucleotide sequence shown in SEQ ID NO:2 may have one or more nucleotide substitutions, insertions or deletions without significantly altering the activity of XBP1. Accordingly, in one embodiment, the polynucleotide sequence encoding XBP1 comprises a sequence having at least 60%, 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 2. In a further embodiment, the invention encompasses the use of polynucleotides that are complementary to, antisense to or hybridize under stringent conditions to the polynucleotide described herein.

The XBP1 employed in the present invention may be derived from any suitable source, for example human, mouse, hamster or yeast.

The cell according to the present invention may be further modified to increase the expression levels of a further component of the unfolded protein response (UPR) pathway relative to the expression levels of the further component and/or modulator of the UPR pathway in an unmodified cell. In one embodiment, the cell further comprises one or more exogenous polynucleotide sequence encoding one or more components and/or modulators of the unfolded protein response (UPR) pathway. Alternatively or additionally, the one or more up-regulated UPR pathway components and/or modulators are from modulation of one or more endogenous polynucleotides encoding the one or more UPR pathway components and/or modulators.

The presence of one or more further components and/or modulators of the UPR pathway may further improve the expression of a protein of interest in a host cell according to the present invention.

In one embodiment the modified cell according to the present invention expresses a protein of interest. "Protein of interest" in the context of the present specification is intended to refer to polypeptide for expression, usually a recombinant polypeptide. However, the protein of interest may be an endogenous protein expressed from an endogenous gene in the host cell.

As used herein, a "recombinant polypeptide" refers to a protein that is constructed or produced using recombinant DNA technology. The protein of interest may be an exogenous sequence identical to the endogenous protein or a mutated version thereof, for example with attenuated biological activity, or fragment thereof, expressed from an exogenous vector. Alternatively, the protein of interest may be a heterologous protein, not normally expressed by the host cell.

The protein of interest may be any suitable protein including therapeutic, prophylactic or diagnostic protein.

The protein of interest expressed by the cells according to the invention may, for example be an immunogenic protein, a fusion protein comprising two heterologous proteins or an antibody. Antibodies for use as the protein of interest include monoclonal, multi-valent, multi-specific, humanized, fully

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human or chimeric antibodies. The antibody may be a complete antibody molecule having full length heavy and light chains or a fragment thereof, e.g. VH, VL, VHH, Fab, modified Fab, Fab', F(ab')₂, Fv or scFv fragment.

After expression antibody fragments may be further processed, for example by conjugation to another entity or for example the antibody fragments may be PEGylated to generate a product with the required properties, for example similar to the whole antibodies, if required.

The cell, one or more polynucleotides, one or more expression cassettes and one or more vectors employed in the present invention may also comprise further polynucleotide sequences encoding one or more further proteins of interest.

The polynucleotides employed in the present invention may be incorporated into the host cell using any suitable means known in the art. Typically, each polynucleotide is incorporated as part of an expression cassette which may be integrated into the chromosome or genome of the host cell or introduced via a non-integrated expression vector. Accordingly, in one aspect the cell according to the present invention comprises an expression cassette comprising one or more polynucleotides encoding Ero1 and XBP1. In one embodiment the cell according to the present invention may comprise an expression cassette comprising both the Ero1 polynucleotide sequence and the XBP1 polynucleotide sequence. In a further embodiment, the cell according to the present invention comprises an Ero1 expression cassette comprising the Ero1 polynucleotide and a separate XBP1 expression cassette comprising the XBP1 polynucleotide sequence.

The polynucleotide sequence encoding a protein of interest may be in the same expression cassette as both XBP1 and Ero1. Alternatively or additionally the polynucleotide sequence encoding a protein of interest may be in the same expression cassette as the Ero1 polynucleotide sequence. Alternatively or additionally the polynucleotide sequence encoding a protein of interest may be in the same expression cassette as the XBP1 polynucleotide sequence. Alternatively or additionally, the cell may further comprise a separate expression cassette comprising the polynucleotide sequence encoding the protein of interest.

In some embodiments, CHOS, CHOSXE, XE4-1, and XE4-1R cells are grown in (CDCHO (Life Technologies), PROCHO5 (Lonza Biowhittiker), MERK (Merck) and PROCHO5/Feed (PROCHO5 and ASF Ajinomoto feed) media. In some embodiments, XE4-1 cells have higher yields of a protein of interest when grown with PROCHO5 or PROCHO5/Feed media than when grown with CDCHO or MERK media. In some embodiments, CHOSXE cells have higher yields of a protein of interest when grown with PROCHO5/Feed media than when grown with PROCHO, CDCHO, or MERK media.

In some embodiments, CHOSXE cells divide more quickly than XE4-1 cells. In some embodiments, XE4-1R cells divide more quickly than XE4-1 cells.

In some embodiments, XE4-1R cells and CHOSXE cells generate relatively equivalent levels of a protein of interest.

The expression cassette employed in the present invention typically comprises one or more protein coding sequences and one or more regulatory expression sequences. The one or more regulatory expression sequences may include a promoter. The one or more regulatory expression sequences may also include a 3' untranslated region such as a polyadenylation sequence or other termination sequences. In one embodiment, each expression cassette comprises a promoter, a protein coding sequence and a polyadenylation sequence. Suitable promoters are discussed in more detail below.

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In the embodiment wherein the expression cassette comprises two or more polynucleotide sequences encoding proteins selected from Ero1, XBP1 and the protein of interest, each coding sequence is operatively linked to one or more suitable regulatory expression sequences to allow expression of each protein.

The expression cassette may further comprise one or more further UPR components and/or modulators, wherein each UPR component and/or modulator is operatively linked to one or more suitable regulatory expression sequences.

In one embodiment, the cell according to the present invention comprises a vector, such as plasmid. The vector preferably comprises one or more of the expression cassettes as defined above. In one embodiment the cell according to the present invention may comprise a vector comprising both the Ero1 polynucleotide sequence and the XBP1 polynucleotide sequence. In a further embodiment, the cell according to the present invention comprises an Ero1 vector comprising the Ero1 polynucleotide and a separate XBP1 vector comprising the XBP1 polynucleotide sequence.

The polynucleotide sequence encoding a protein of interest may be in the same vector as both XBP1 and Ero1. Alternatively or additionally the polynucleotide sequence encoding a protein of interest may be in the same vector as the Ero1 polynucleotide sequence. Alternatively or additionally the polynucleotide sequence encoding a protein of interest may be in the same vector as the XBP1 polynucleotide sequence. Alternatively or additionally, the cell may further comprise a separate vector comprising the polynucleotide sequence encoding the protein of interest.

In the embodiments of the present invention wherein a polynucleotide sequence comprises two or more encoding sequences for two or more proteins selected from Ero1, XBP1, one or more further UPR components and/or modulators and one or more proteins of interest, the polynucleotide sequence may comprise one or more internal ribosome entry site (IRES) sequences which allows translation initiation in the middle of mRNA. An IRES sequence may be positioned between encoding polynucleotide sequences to allow separate translation of the mRNA to produce the encoded polypeptide sequences.

The vector for use in the present invention may be produced by inserting an expression cassette as defined above into a suitable vector. Alternatively, the regulatory expression sequences for directing expression of each polynucleotide sequence encoding a protein may be contained in the vector and thus only the encoding region of the one or more polynucleotides may be required to complete the vector.

The vectors employed in the present invention are capable of accommodating one or more polynucleotide sequences encoding proteins selected from Ero1, XBP1, the protein of interest and optionally one or more further UPR components and/or modulators.

Examples of vectors which may be employed to transfect the host cell with a polynucleotide according to the invention include:

a plasmid, such as an expression vector, and/or a viral vector such as bacterial phage, lentiviral, episomal eg BPV, vaccina, SV40.

An example of a suitable starting vector is pcDNA3 (Invitrogen).

The promoters employed in the present invention can be linked to the relevant polynucleotide directly or alternatively be located in an appropriate position, for example in a vector such that when the relevant polypeptide is inserted the relevant promoter can act on the same. One promoter may be employed for all the encoded sequences but greater levels of

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expression are generally obtained when each polypeptide encoded has a specific promoter.

Therefore in one embodiment one, two, three or more promoters are employed.

In one embodiment one or more promoters are located in the polynucleotide employed in the invention.

In one embodiment the promoter is located before the encoding portion of the polynucleotide on which it acts, for example a relevant promoter before each encoding portion of polynucleotide. "Before" as used herein is intended to imply that the promoter is located at the 5 prime end in relation to the encoding polynucleotide portion.

The promoters employed in the present invention may be the same or different for each polynucleotide. The promoters may be endogenous or exogenous to the host cells. Suitable promoters include CMV such as hCMV (for example suitable as a promoter when the polypeptide is an antibody), viral LTR promoters and SV40 promoter.

One or more promoters employed may be inducible promoters.

In one embodiment, the polynucleotide employed in the present invention comprises a polyadenylation signal sequence, for example, associated with each polynucleotide sequence encoding a protein selected from Ero1, XBP1 and the protein of interest as appropriate, such as at the end of (for example at the C-terminal (3 prime) end of) each encoding polynucleotide. Expression vectors, in particular, may require signal sequences that encode for a polyadenylation tail. The polyadenylation signal sequence causes a polyadenylation tail to be added at the end of the transcribed pre-mRNA that may protect the mRNA from exonucleases thereby stabilizing mRNA and may terminate transcription.

Examples of polyadenylation tails include SV40poly A, BgH polyA and synthetic polyA tail, in particular SV40 poly A.

In one embodiment the polynucleotide employed in the present invention comprises one or more introns. In one embodiment the polynucleotide sequence comprises an intron before the start codon, i.e. at the 5 prime end. In one embodiment the polynucleotide sequence comprises an intron after the stop codon, i.e. at the 3 prime end. In one embodiment the polynucleotide sequence comprises 1, 2 or 3 introns. In one embodiment the polynucleotide sequence comprises an intron before the start codon and after the stop codon. The intron may be derived from any gene, particularly a gene from which the encoded sequence is derived.

In one or more embodiments the polynucleotide employed in the present invention comprises a Kozak sequence associated with each polynucleotide sequence encoding a protein selected from Ero1, XBP1 and the protein of interest as appropriate. Whilst not wishing to be bound by theory it is thought that the Kozak sequence is an optimal ribosome binding site.

Embodiments of the invention described herein with reference to the polynucleotide apply equally to alternative embodiments of the invention, for example vectors, expression cassettes and/or host cells comprising the components employed therein, as far as the relevant aspect can be applied to same.

The present invention also provides a method comprising modifying a cell to increase the capability of the cell to increase the expression levels of Ero1 and XBP1 relative to the expression levels of Ero1 and XBP1 in an unmodified cell.

The increase of Ero1 and XBP1 may be performed using any suitable means known in the art. For example, the cell

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may be transfected with one or more polynucleotides, one or more expression cassettes and/or one or more vectors encoding:

- (1) an exogenous polynucleotide sequence encoding Ero1 or a variant thereof which substantially retains the function of Ero1; and
- (2) an exogenous polynucleotide sequence encoding XBP1 or a variant thereof which substantially retains the function of XBP1.

In this embodiment the one or more polynucleotides, one or more expression cassettes and/or one or more vectors are as described above. Accordingly, the method according to the present invention may employ the polynucleotide according to the present invention wherein the polynucleotide comprises a polynucleotide sequence comprising Ero1 and a polynucleotide sequence comprising XBP1. Additionally or alternatively, the method according to the present invention employs separate polynucleotides encoding Ero1 and XBP1, as described above.

In an additional or alternative embodiment, Ero1 and XBP1 expression is increased from modulation of endogenous polynucleotides encoding Ero1 and XBP1 in the cell. In this embodiment the cell is modified to increase transcription and translation of the endogenous genes of Ero1 and XBP1. Any suitable method to increase expression of endogenous genes may be used. For example, an agent, such as Tunicamycin or Thapsigargin, may be used to cause cell stress and thereby stimulating UPR and Ero1 and XBP1 increase. A further example is the use of an agent, such as CpG binding protein (a transcriptional activator that binds unmethylated CpG motifs), may be used to enhance the transcription of genes including Ero1 and XBP1.

The one or more polynucleotides employed in the present invention, preferably in the form of one or more expression cassettes or one or more vectors, can be incorporated into a cell in various ways. In one embodiment the one or more polynucleotides are integrated into a chromosome or the genome of the cell to allow stable expression. In a further embodiment of the invention the cell is transiently transfected using, for example a non-integrating vector.

In the embodiment wherein the Ero1 polynucleotide sequence and XBP1 polynucleotide sequence are separate, each polynucleotide sequence, preferably in the form of an expression cassette or vector, may be introduced into a cell simultaneously or sequentially.

The one or more polynucleotide sequences can be introduced into a cell using standard techniques, for example employing electroporation, or lipid based methods (lipotransfection), anionic transfection, cationic transfection such as employing calcium phosphate, heat shock, magnetofection, transfection agents such as lipofectamine, dendrimers, DEAE-dextran transfection, transduction employing a virus. In one embodiment cationic transfection such as employing calcium phosphate is employed.

The method according to the present invention may also employ a selection system to facilitate selection of stable cells which have been successfully transfected with the one or more polynucleotides. The selection system typically employs co-transfection of a polynucleotide sequence encoding a selection marker. In one embodiment, each polynucleotide transfected into the cell further comprises a polynucleotide sequence encoding one or more selection markers. Accordingly, the transfection of the one or more polynucleotides of the present invention and the one or more polynucleotides encoding the marker occurs together and the selection system can be employed to select those cells which produce the desired proteins.

Cells able to express the one or more markers are able to survive/grow/multiply under certain artificially imposed conditions, for example the addition of a toxin or antibiotic, because of the properties endowed by the polypeptide/gene or polypeptide component of the selection system incorporated therein (e.g. antibiotic resistance). Those cells that cannot express the one or more markers are not able to survive/grow/multiply in the artificially imposed conditions. The artificially imposed conditions can be chosen to be more or less vigorous, as required.

Any suitable selection system may be employed in the present invention. Typically the selection system may be based on including in the vector one or more genes that provides resistance to a known antibiotic, for example a kanamycin or ampicillin resistance gene. Cells that grow in the presence of a relevant antibiotic can be selected as they express both the gene that gives resistance to the antibiotic and the desired protein. Geneticin, also known as G418, is a toxin that can be neutralized by the product of a neomycin resistant gene. In some embodiments, a vector comprising Ero1, XBP1, or a combination thereof also comprises a G418 selection system comprising the neomycin phosphotransferase resistance gene. Other suitable selection systems include the use of the enzyme dihydrofolate reductase (DHFR), which is essential for the de novo synthesis of glycine, purine and thymidine, optionally in combination with an inhibitor of DHFR namely, methotrexate, and the use of glutamine synthetase (GS), which catalyses the formation of glutamine from glutamate and ammonia, optionally in combination with an inhibitor of GS, such as methionine sulfoximine (MSX). The Zeocin™ selection system may also be employed in the present invention. Zeocin™ is an antibiotic from *Streptomyces* and the selection system employs the use of a Zeocin™ resistant protein.

In one embodiment, the method according to the present invention further comprises the step of culturing the transfected cell in a medium to thereby express increased levels of Ero1 polypeptide and XBP1 polypeptide and optionally any selection marker.

In this embodiment the method according to the present invention may further comprise the following steps:

- a) selecting one or more modified cell clones;
- b) measuring the quantity of Ero1 mRNA and/or protein in the selected one or more cell clones; and
- c) measuring the quantity of XBP1 mRNA and/or protein in the selected one or more clones.

Steps b and c may be carried out simultaneously or sequentially in any order. The skilled person would know from their common general knowledge suitable method for measuring the mRNA and/or protein levels of Ero1 or XBP1 in modified cell clones. Techniques for measuring the increase of Ero1 and XBP1 expression include ELISA for protein quantification and Taqman quantitative PCR analysis and Northern Blot analysis for mRNA quantification.

The method preferably further comprises the steps of:

- d) comparing the quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein to the quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein in an unmodified cell clone; and
- e) selecting one or more modified cell clones having a higher quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein compared to the unmodified cell clone.

In step d the quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein is compared to the quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein in an unmodified cell clone. Alternatively the quantity of Ero1

mRNA and/or protein and XBP1 mRNA and/or protein may be compared to the quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein in one or more other modified cell clones. In this embodiment, step e may comprise selection of the modified cell clones showing a higher quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein compared to other modified cell clones.

In step e, any suitable number of modified cell clones may be selected which have higher quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein compared to the unmodified cell clone. Typically one or more cell clones having the highest quantity of Ero1 mRNA and/or protein and XBP1 mRNA and/or protein are selected. The one or more cell clones which are selected in step e may then be selected for further growth and use for expressing a protein of interest. The method may comprise repeating steps b, c, d and e one or more times.

The skilled person would easily be able to identify modified cell clones having a higher quantity of Ero1 mRNA and XBP1 mRNA compared to the unmodified cell clone, such as a CHOS cell. Examples of suitable relative quantity of Ero1 mRNA and XBP1 mRNA compared to a CHOS cell which has no detectable Ero1 mRNA and XBP1 mRNA (which is counted as 1 for the purpose of calculating relative amount), may be for Ero1 mRNA: 5000 or more fold increase, 5000 to 500000 fold increase, 10000 to 400000 fold increase, 30000 to 400000 fold increase, 100000 to 400000 fold increase; and for XBP1 mRNA: 150 or more fold increase, 150 to 150000 fold increase, 300 to 110000 fold increase, 5000 to 110000 fold increase.

The present invention also provides one or more modified cell clones as produced by the method described above. The present inventors have found that the modified cell clones produced by the above method are capable of expressing a protein of interest at a higher yield compared to an unmodified cell clone.

In one embodiment the method further comprises transfecting the host cell with a polynucleotide sequence encoding a protein of interest and expressing the protein of interest. The method may also include a further step of measuring the quantity of expression of the protein of interest and selecting one or more cell clones having high expression levels of the protein of interest. Accordingly, the present invention also provides a method of producing a recombinant protein of interest comprising expressing the recombinant protein of interest in a modified host cell in which the expression levels of XBP1 and Ero1 are increased. Suitable proteins of interest are described above.

In the embodiment wherein the cell is also transfected with a polynucleotide sequence encoding a protein of interest which is a separate polynucleotide to the one or more Ero1 and XBP1 polynucleotides, the polynucleotide sequence encoding a protein of interest may be introduced into the host cell simultaneously with the one or more Ero1 and XBP1 polynucleotides, wherein the Ero1 and XBP1 polynucleotide sequences may be in the same or separate polynucleotides. Alternatively, the polynucleotide sequence encoding a protein of interest may be introduced into the host cell before or after the one or more Ero1 and XBP1 polynucleotides are introduced into the host cell. In the embodiment wherein the Ero1 and XBP1 polynucleotide sequences are separate polynucleotides the polynucleotide sequence encoding a protein of interest may be introduced into the host cell before or after one or both of the Ero1 and XBP1 polynucleotides.

In a preferred embodiment the polynucleotide sequence encoding a protein of interest is introduced into the host cell after the one or more Ero1 and XBP1 polynucleotides have

been introduced into the host cell and, more preferably the polynucleotide sequence encoding a protein of interest is introduced into the host cell after the modified cells have been selected which show increased Ero1 and XBP1 expression.

An inducible expression system may be used in the present invention to express the Ero1 protein and/or the XBP1 protein and/or the protein of interest. Suitable inducible expression systems are well known in the art.

In one embodiment wherein the cell comprises a polynucleotide sequence encoding a further component and/or modulator of the UPR pathway, the method may further comprise a step of expressing the further component and/or modulator of the UPR pathway.

Any suitable medium may be used to culture the transfected cell. The medium may be adapted for a specific selection system, for example the medium may comprise an antibiotic, to allow only those cells which have been successfully transfected to grow in the medium. The method according to the present invention may also comprise a step of selecting those cells in the medium which have been successfully transfected, such as by selecting cells which are able to grow in the medium.

The cells obtained from the medium may be subjected to further screening and/or purification as required. In the embodiment wherein the cell comprises a polynucleotide sequence encoding a protein of interest and the protein of interest is expressed, the method may further comprise one or more steps to extract and purify the protein of interest as required.

One or more method steps described herein may be performed in combination in a suitable container such as a bioreactor.

The present invention also extends to cell cultures that are transfected or transduced with one or more polynucleotides, as described herein, preferably in the form of one or more expression cassettes or one or more vectors, as described above.

The present inventors have demonstrated that the co-expression of both Ero1 and XBP1 in a cell provides improved means for expressing a protein of interest.

As discussed above, with respect to the cell according to the present invention, in a preferred embodiment the method according to the present invention increases the yield of expressed protein of interest from the cell compared to the yield of expressed protein of interest from an unmodified cell which has not been modified to increase either Ero1 or XBP1, more preferably compared to the yield of expressed protein of interest from a cell which has been modified to increase either Ero1 or XBP1 and still more preferably compared to the combined yield of expressed protein of interest from a cell modified to increase the expression of Ero1 but not XBP1 and a cell modified to increase XBP1 but not Ero1.

Further, as discussed previously, the method according to the present invention preferably provides a cell having increased cell growth and/or stability and/or reproduction after the same period of culture relative to unmodified cells which have not been modified to increase Ero1 and XBP1.

The present invention also provides an isolated polynucleotide comprising a polynucleotide sequence encoding Ero1 or a variant thereof which substantially retains the function of Ero1; and a polynucleotide sequence encoding XBP1 or a variant thereof which substantially retains the function of XBP1. Suitable polynucleotide sequences including preferred sequences are described above with respect to the cell according to the present invention, wherein both Ero1 and XBP1 are part of the same polynucleotide sequence. In one embodiment, the polynucleotide according to the present

invention may further comprise a polynucleotide sequence encoding a protein of interest and/or a further component of the UPR pathway, as described previously.

The present invention also provides an expression cassette and a vector comprising the polynucleotide according to the present invention.

The invention also extends to an expression system comprising a recombinant polynucleotide sequence encoding Ero1 or a variant thereof which substantially retains the function of Ero1; a recombinant polynucleotide sequence encoding XBP1 or a variant thereof which substantially retains the function of XBP1; and a suitable medium.

The expression system may comprise a polynucleotide comprising both Ero1 and XBP1 or may comprise separate polynucleotides comprising Ero1 and XBP1. The one or more polynucleotides are preferably comprised within one or more expression cassettes and/or one or more vectors as described previously. The expression system may comprise a cell suitable for transfecting with the one or more polynucleotide. Thus the invention provides a composition comprising a polynucleotide according to the invention and a cell complementary thereto, for example in admixture and/or wherein the relevant polynucleotide is located within the cell. In one embodiment the expression system comprises a cell comprising the one or more polynucleotides as defined above.

The expression system may further comprise one or more polynucleotides encoding different proteins of interest.

The invention also extends to use of a recombinant polynucleotide sequence encoding Ero1 and a recombinant polynucleotide sequence encoding XBP1 for expressing a protein of interest.

In a further aspect the invention provides use of a recombinant polynucleotide sequence encoding Ero1 and a recombinant polynucleotide sequence encoding XBP1 in one or more expression cassettes, one or more vectors or one or more cells as described above for expressing a protein of interest.

The present invention also provides a polynucleotide as defined above, an expression cassette as defined above, a vector as defined above, a cell as defined above or an expression system as defined above for use as a medicament.

The present invention also encompasses a pharmaceutical composition comprising a polynucleotide as defined above, an expression cassette as defined above, a vector as defined above, a cell as defined above or an expression system as defined above.

The present invention also encompasses a composition comprising Ero1 protein and XBP1 protein for use as a medicament. The present invention also encompasses a pharmaceutical composition comprising Ero1 protein and XBP1 protein.

Suitable pharmaceutically acceptable carriers including solvents, solubilizers, fillers, stabilizers and the like are well known in the art. The pharmaceutical composition may be formulated for any intended route of administration including but not limited to parental, intravenous, oral, inhalation, topical and systemic administration. In one embodiment the one or more expression cassettes or one or more vectors according to the present invention are suitable for gene delivery for being introduced into a patient. In this embodiment the vector is preferably a viral vector. The one or more expression cassettes or one or more vectors may be encapsulated in a liposome for gene delivery. Various other methods of introducing genes into a patient are well known in the art.

The over-expression of both Ero1 and XBP1 or a composition comprising the combination of both Ero1 and XBP1 may be used to treat protein conformational diseases or disorders. Accordingly, the present invention also provides the

use of a polynucleotide as defined above, an expression cassette as defined above, a vector as defined above, a cell as defined above, an expression system as defined above or a pharmaceutical composition as defined above for manufacture of a medicament for the treatment of a protein conformational disease or disorder. The increase of Ero1 and XBP1 may be used to treat conditions wherein Ero1 and/or XBP1 expression or activity is reduced. Typical protein conformational diseases which may be treated include, but are not limited to cystic fibrosis, α 1-antitrypsin deficiency and autoimmune diseases.

In a further embodiment, wherein a polynucleotide sequence encoding a protein of interest is employed in the present invention, the protein of interest may be a therapeutic or prophylactic protein suitable for the treatment of a human or non-human animal in need thereof. In some embodiments, CHOSXE cells or XE4-1R cells are for use as a medicament.

One or more embodiments of the invention described herein may be combined unless they are technically incompatible.

Specific embodiments of the invention are described herein comprising certain entities. The invention also extends to separate embodiments that consist or consist essentially of said elements.

The invention will now be illustrated by reference to the following examples.

EXAMPLES

DNA Manipulations and General Methods

CHOS cells (Invitrogen) and CHOK1 cells (ATCC) were used for transformation and routine culture growth. DNA restriction and modification enzymes were obtained from New England Biolabs.

The spliced form of human XBP1 (SEQ ID NO:2) and Human Ero1 α (SEQ ID NO:4) were used.

Nucleotide sequences for Ero1 α and XBP1 were chemically synthesized by Entelechon.

The pCDNA3.1(+) vectors comprising the gene of interest, were transfected into the host cells using electroporation (for CHOSX and CHOK1E cells) or lipofectamine (for CHOSXE cells).

In example 2 and 3 the mammalian expression vectors used contained both sequences encoding the heavy and light chain of an antibody. These sequences were under control of the CMV-promoter. The plasmid also included SV40E polyA tails and the glutamate synthetase selection marker.

In example 5 and 6 single gene vectors were used containing sequences encoding either the heavy or the light chain of the monoclonal antibody. No selection marker was present on the plasmid. The single gene vectors were co-transfected at a 1:1 ratio.

Lipofectamine transfections were done using Lipofectamine2000 (Invitrogen). 12.5 μ l Lip2000 was diluted into 125 μ l serum free Dulbecco's modified Eagle's media (SF DMEM, Invitrogen). 4 μ g of the mAb expression vector DNA was diluted into 125 μ l SF DMEM. DNA and Lip2000 were mixed and incubated for 20 minutes at room temperature. 500 μ l SF DMEM was added to the mix which was then added to the cells to incubate at 37° C. for four hours. Subsequent to incubation, the media containing the DNA/reagent mix was replaced by 3 ml of fresh CDCHO media (Invitrogen). MAb transfections were done in duplicate.

Antibodies used in the examples were IgG antibodies of the following isotypes show in Table 1 below:

TABLE 1

mAb	Species	Isotype	Extinction Coefficient
146	Mouse	IgG1	
632	Mouse	IgG2a	
497	Human	IgG4	
240	Human	IgG4	
42	Mouse	IgG1	1.6
61	Mouse	IgG1	1.62
164	Mouse	IgG1	1.6

Levels of antibody expression were determined by ELISA unless otherwise stated.

Cell density and cell viability was measured using CEDEX (Innovatis) automated cell counting system based on the well-established Trypan Blue exclusion method for determining cell viability. Sample handling, staining, cell counting and graphical analysis of the results are performed automatically by the CEDEX system.

Example 1

Transfection of CHO Cells with Ero1 α and/or XBP1

CHOSX Cell Line

The spliced form of human XBP1 [hXBP1(s)] was cloned into vector pCDNA3.1(+), this vector also contained the G418 selection system comprising the neomycin resistance gene, which generated the vector pCDNA3.hXBP1(s). CHOS cells were transfected with vector pCDNA3.hXBP1(s) and stable cell lines were selected in the presence of geneticin (1 mg/ml) to produce a CHOSX cell line expressing hXBP1(s).

CHOK1E Cell Line

Human Ero1 α (hEro1 α) was cloned into the pCDNA3.1(+) vector and CHOK1 cells were transfected with pCDNA3.hEro1 α to produce a CHOK1E cell line expressing human Ero1 α . CHOK1E cells were selected using the G418 selection system.

CHOSXE Cell Line

Human Ero1 α (hEro1 α) was cloned into vector pCDNA3.1(+) (zeo) (Invitrogen) and the CHOSX cell line was then supertransfected with vector pCDNA3(zeo).hEro1 α to produce the CHOSXE cell line expressing both hEro1 α and hXBP1(s). Both the G418 selection system (neomycin resistance gene) and the zeocin selection system (zeocin resistance gene) were used to select successfully transfected CHOSXE cells. The cells were grown in 100-300 μ g/ml of zeocin and 1 mg/ml of geneticin.

Selection of CHOSXE Clones

Pick 1:

Two weeks post transfection a first pick of 177 clones was performed of which 85 clones were positive for mRNA expression of the transgenes hEro1 α and hXBP1(s) as assessed by single cell RT-PCR and PCR.

24 of the 85 positive clones were then bulked up to 6-well plates. Relative mRNA levels of hXBP1(s) and hEro1 α were determined by Taqman. The results of this analysis are shown in FIGS. 3a and 3b where the relative hXBP1(s) and hEro1 α mRNA levels in the CHOSXE clones are compared to CHOS cells (CHOS cells showed no expression but counted as 1 for the purpose of calculating relative mRNA amounts). Using the results shown in FIGS. 3a and 3b, 3 low hEro1 α expressing clones (70, 77 and 103), 3 medium hEro1 α expressing clones (76, 88 and 173) and 3 high hEro1 α expressing clones (86, 94 and 101) were then selected for further analysis.

Pick 2:

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A further two weeks after pick 1 was performed 72 more clones were picked of which 29 clones were positive for hXBP1(s) and hEro1 α mRNA expression as assessed by RT-PCR and PCR.

The 29 positive clones were then bulked up to 6-well plates and relative levels of hXBP1(s) and hEro1 α mRNA were determined as in pick 1. The results of this analysis are shown in FIG. 4 where the mRNA levels of hXBP1(s) and hEro1 α expressed in the CHOSXE clones are compared to CHOS cells (CHOS cells showed no expression but counted as 1 for the purpose of calculating relative mRNA amounts). Further mRNA analysis of clone 71 (data not shown) showed mRNA levels of 5,000 fold increase of hXBP1(s) and 110,000 fold increase of hEro1 α compared to CHOS cells (CHOS cells showed no expression but counted as 1 for the purpose of calculating relative mRNA amounts). Using the results shown in FIG. 4, 3 low hEro1 α expressing clones (19, 23, and 64), 3 medium hEro1 α expressing clones (13, 16 and 62) and 3 high hEro1 α expressing clones (49, 57 and 71) were then selected for further analysis.

Clone 71 was deposited under Depositor Deposit Name: CHOSXE (Depositor Identification in Full: CHOS.Xbp1.Ero1a), on 10 Feb. 2010 by UCB Celltech, the UK branch of UCB Pharma S.A., at the European Collection of Cell Cultures (ECACC), HPA, United Kingdom, under HPA Culture Collections Reference Number: Q8515 and Accession number: 10021001 in accordance with The Budapest Treaty.

Example 2

Transfection of CHOSXE Cells with Monoclonal Antibodies (mAbs)

The selected clones from pick 1 (70, 76, 77, 86, 88, 94, 101, 103 and 173) were transfected with DNA of mAbs 146 and 632 as described in the methods and the mAb expression was determined from culture supernatant. The 3 highest mAb expressing CHOSXE clones were selected for further analysis (clones 70, 88 and 103).

The three selected clones from pick 1 (70, 88, and 103) were then combined with the selected clones from pick 2 (13, 16, 19, 23, 49, 57, 62, 64, and 71) and transfected with DNA for mAbs 632 and 497 as described in the general methods section above. Culture supernatant sample was taken on day 4 to determine antibody concentration.

Monoclonal antibodies 632 and 497 were also transfected into CHOS and CHOSX cells using the same transfection method as described in the general methods section above.

The data for the relative increase in expression of mAbs 632 and 497 from CHOSXE and CHOSX cells compared to the expression of mAbs 632 and 497 from CHOS cells is shown in FIG. 5. The data indicates that all the CHOSXE clones, except for clone 13 and clones 23, produced increased mAb expression levels of mAb 632 compared to the CHOS clone and all the CHOSXE clones produced increased expression of mAb 497 compared to both the CHOS the CHOSX clones, except for clone 23 which expressed less mAb 497 than CHOSX.

Example 3

Transfection of CHOSXE Cells with Antibodies Ab240 and Ab146

The highest mAb expressing clones (16, 19, 57, 62, 71 and 88) produced in Example 2 were picked to perform further

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mAb transfections. The experiment was scaled up to T25 flask cultures and the mAb 240 and 146 were used. The transfection was performed as described above in the general methods section, but 18.75 μ l Lip2000 was diluted into 1.875 ml OPTI_MEM (Invitrogen) media. 7.5 μ g of antibody DNA was diluted into 1.875 ml OPTI_MEM media. 3.75 ml of OPTI_MEM media was added to the mix to add to the cells. After incubation at 37° C., 3.375 ml CD CHO media was added to the flask. This transfection method was used for CHOSXE cells, CHOSX cells, CHOK1E cells and CHOS cells.

The data for the increase in expression of mAbs 240 and 146 from CHOSXE, CHOSX and CHOK1E cells compared to the expression of mAbs 240 and 146 from CHOS cells is shown in FIG. 6. In FIG. 6 clones numbered 16, 19, 57, 62, 71 and 88 are CHOSXE clones. The data indicates that all the CHOSXE clones produced increased expression of both antibodies compared to CHOS cells, CHOSX cells and CHOK1E cells.

Clone 71 was selected for further analysis and is called CHOSXE in Examples 4 to 7.

Example 4

Measurement of Cell Line Characteristics in CHOSXE, CHOSX and CHOS Cell Lines

Cells from cell lines CHOSXE, CHOSX and CHOS were seeded at 2×10^5 /ml in 50 mls CD CHO and cultured over 7 days on a shaking platform. As shown in FIG. 7, the CHOSXE cell line outperformed the CHOS, CHOSX, CHOK1 and CHOK1E cell lines by reaching higher viable cell densities.

Example 5

Electroporation of Antibodies 42, 61 and 164 into CHOSXE, CHOSX and CHOS Cell Lines

400 μ g DNA of an antibody was transfected into 3 different cell lines CHOS, CHOSX and CHOSXE. Cells were shifted to 32° C. on day 1 and 3 μ M NaBu added on day 4 post-transfection. The three antibodies used were labelled as mAbs 42, 61 and 164. Cell counts and cell viabilities were taken on day 1, 4, 7 and 10. Culture supernatant samples were taken on days 4, 7 and 10 to determine cells' specific productivity.

TABLE 2

% Cell viabilities and viable cell densities (VCD) on day 1 post-transfection.				
Antibody	Cell Line	VCD cells $\times 10^5$ /ml (day 1)	% Viability (day 1)	
42	CHOS 42	22.7	96.7	
	CHOSX 42	23.85	96.8	
	CHOSXE 42	23.05	97.1	
61	CHOS 61	23.4	96.8	
	CHOSX 61	20.14	96.9	
	CHOSXE 61	23.54	97.8	
164	CHOS 164	20.92	97.5	
	CHOSX 164	22.04	96.5	
	CHOSXE 164	23.73	97.5	

It can be seen from Table 2 that CHOXE cell lines had equal or higher % viability compared to CHOS and CHOSX and that CHOSXE had comparable viable cell densities compared to CHOS and CHOSX.

FIG. 8 shows the relative increase in expression of mAbs 42, 61 and 164 from CHOSXE and CHOSX compared to the expression of mAbs 42, 61 and 164 from CHOS cells. It is clear that expression of all three antibodies was higher in CHOSXE compared to either CHOSX or CHOS.

The specific productivity rate (SPR) of each cell line was also calculated. FIG. 9 shows the SPR of CHOS, CHOSX and CHOSXE cell lines in $\mu\text{g}/1 \times 10^6$ cells/day between day 4 and day 7. Specific productivity rate per cell was clearly higher in CHOSXE compared to either CHOS or CHOSX cell lines.

The relative mRNA levels of heavy and kappa light chains of the mAbs 42, 61 and 164 were also measured by real time PCR (Taqman). The results are shown in FIG. 10, it can be seen that CHOSXE produced higher levels of the heavy and light chain mRNA compared to CHOS and CHOSX.

Example 6

Electroporation of Antibodies into CHOSXE, CHOSX, CHOS and CHOK1 Cell Lines and Subsequent Purification

CHOS, CHOSX, CHOSXE and CHOK1 cells were transfected as outlined in example 5.

Mab quantification was done by high-performance liquid affinity chromatography (HPLC) utilizing Protein G as a ligand. A standard curve was generated by injecting known amounts of purified IgG and analyzing the elution peaks. mAb eluate was monitored at 280 nm and extrapolated from the standard curve to calculate the mAbs' concentrations using their respective extinction coefficients (concentration=absorbance/extinction coefficient) (Table 1).

mAbs were purified using MabSelect Sure columns. The protein-A derived MabSelect Sure ligand is derived from *E. coli* and has been engineered to create an affinity medium with enhanced alkali stability and high binding capacity for IgG. At this point a trace HPLC was done to determine the percentage aggregate of the affinity-purified pooled fractions. The degree of aggregation exhibited by mAbs expressed in CHOSXE was comparable to CHOS, CHOSX and CHOK1 (see FIG. 11). Following MabSelect Sure, antibodies were further purified using size exclusion HPLC. The total mAb yield post-purification was also determined by reading absorbance of the mAb at 280 nm and calculating concentration as above.

Table 3 shows increases in antibody concentration relative to the CHOS cells after prot G HPLC and protein purification.

TABLE 3

		Fold increase	
		Prot G HPLC	Yield after Purification
Ab42	CHOS	1.00	1.00
	CHOSX	1.09	0.83
	CHOSXE	6.28	7.22
	CHOK1	1.65	2.70
Ab61	CHOS	1.00	1.00
	CHOSX	2.39	3.30
	CHOSXE	5.36	5.24
	CHOK1	1.58	2.35
Ab164	CHOS	1.00	1.00
	CHOSX	2.68	2.44
	CHOSXE	5.94	6.14
	CHO K1	1.94	2.80

It can be seen from Table 3 that the CHOSXE cells produced a significantly higher quantity of antibody compared to CHOS, CHOSX and CHOK1 cells.

Example 7

Antibody Quality Analysis

Purified mAbs from CHOSXE cells were analyzed for quality and activity as described below.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

MAbs 42, 61 and 164 were analyzed by SDS PAGE to determine whether the expressed antibody was of the expected size. The SDS PAGE analysis was performed by running 5 μg of reduced and non-reduced protein on a pre-packed 4-12% Bis-Tris gradient gel (Invitrogen) at 50 mA for 45 minutes. FIGS. 12a, b and c show the results of the SDS PAGE, it can be seen that the antibodies were the same size whether expressed from CHOS, CHOSX, CHOSXE or CHOK1.

Thermofluor Assay

The thermostability of MAbs 42, 61 and 164 were analyzed in a Thermofluor assay. This assay allows one to determine at which temperature the protein structure becomes unfolded. An environmentally sensitive fluorescent dye was used to monitor the thermal unfolding process of the proteins. The dye binds hydrophobic regions that become exposed upon unfolding and changes its emission spectrum. 1 μl of sample at 1 mg/ml, 1 μl of 30 \times dye, and 8 μl of buffer was placed into a well of a 384 PCR optical well plate (samples run in quadruplicate). 7900HT fast real-time PCR system contains a heating device for accurate temperature control set at 20 $^{\circ}$ C. to 99 $^{\circ}$ C., a CCD device simultaneously monitors the fluorescence changes in the wells. Standard deviations were less than 0.5 $^{\circ}$ C., for the thermofluor assay data presented. The results from the Thermofluor assay are shown in FIG. 13 where it can be seen that there were no significant differences between the thermostability properties of mAbs when expressed from CHOS or CHOSXE.

Iso-Electric Focussing

MAB 42, 61 and 164 were analyzed by Iso-electric focusing to determine if the expressed antibodies had the same net charge. High voltage was applied across the capillary using anode and cathode, which were dipped, in small reservoirs containing catholyte (OH^-) and anolyte (H^+). Samples were prepared with carrier ampholytes and on application of high voltage the protein molecules migrated and focused according to their respective pIs. The system used was the iCE 280 from Convergent biosciences (Isogen in Europe) which is an imaged capillary isoelectrophoresis instrument used to determine pIs of various protein samples and their related species. The results showed no major differences between the charges antibodies expressed in CHOK1SV, CHOS and CHOSXE.

Mass Spectrometry

The glycosylation profiles of mAbs 42, 61 and 164 were analyzed by mass spectrometry. 100 μg of each sample was treated with PNGase under native conditions to release N-glycans. Glycan separated from deglycosylated protein by SEC and the glycan fraction was analysed by MALDI Mass

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Spec. This analysis determined if the expressed antibodies had the same glycosylation profiles and whether any unfavourable sugar moieties had bound to the antibodies. The results from the glycosylation profiles are shown in FIG. 14 show that there were no significant differences between the glycosylation profiles of the antibodies expressed from CHOS and CHOSXE.

Antigen Binding Assay

MAb164 was analyzed in an antigen binding assay to determine if the expressed antibody had the same antigen binding affinity. BALB/C splenocytes expressing the target antigen of mAb 164 were treated with a titration of mAb 164. Cells were washed and treated with a PE labeled anti-mouse IgG1. Fluorescence cell staining was determined using FACS. The results from the assay are shown in FIG. 15 where "Geo mean" is the geometric mean value of fluorescence of antibody binding. MOPC21 is anti-mouse IgG1 antibody and used as a negative control. FIG. 15 shows that mAb 164 expressed from CHOSXE did not have significantly different antigen binding activity compared to the antibody expressed from CHOS.

In view of the above, it is clear that the antibodies expressed from CHOSXE did not exhibit any major differences from the antibodies expressed from other cell lines. Accordingly, the cell according to the present invention is capable of expressing a protein of interest, such as an antibody, having the same properties compared to when the protein is expressed in other cells, specifically cells which have not been modified to over express Ero1 and XBP1.

Example 8

Transfection of CHO Cells with Ero1 α and XBP1

The spliced form of human XBP1 [hXBP1(s)] was cloned into a multiple cloning site located between a CMV promoter and SV40PolyA in a mammalian expression vector. Human Ero1 α (hEro1 α) was cloned into a multiple cloning site located between a second CMV promoter and SV40polyA, ensuring both hXBP1(s) and hEro1 α cDNAs were housed within their own cassette in the same vector. This vector also comprises the G418 selection system comprising the neomycin phosphotransferase resistance gene.

This vector containing the hXBP1(s) and hEro1 α cDNA was transfected into Freedom CHOS cells (Life technologies) by electroporation. Transfected cells were plated at 500, 1000, or 2500 cells/well in a 96 well plate supplemented with 1 mg/ml Geneticin.

Selection of Cell Clones

Clones (48) were randomly selected from the transfected cells plated at 500 cells/well and were analysed for increased levels of hEro1 α mRNA using RT-PCR. 24 wells positive for hEro1 α mRNA were cultured to a larger 15 ml scale and frozen. Sixteen cell lines were analysed for hXBP1(s) and hEro1 α using Taqman analysis (FIG. 16). A number of cell lines were analysed for antibody yield; the cell line yielding the highest level of hEro1 α , named XE4-1, was chosen. This cell line was sub-cultured to improve its growth capabilities and renamed XE4-1R.

Taqman was used to analyse the levels of hXBP1(s) and hEro1 α for XE4-1, XE4-1R, CHOSXE, and the host CHOS parental cell line. The results are shown in FIG. 17. The data shows that the mRNA level of hXBP1(s) and hEro1 α were elevated in XE4-1 cells compared to the parental CHOS cells.

Clone XE4-1R was deposited under Depositor Deposit Name: XE4-1R on 25 Mar. 2014 by UCB Celltech, the UK branch of UCB Pharma S.A., at the European Collection of

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Cell Cultures (ECACC), HPA, United Kingdom, under Accession number: 14032501 in accordance with The Budapest Treaty.

Example 9

Transfection of CHOS, XE4-1 and CHOSXE Cells with Monoclonal Antibodies (mAbs)

2×10^9 XE4-1 cells/ml were transfected with 4 mg of antibody DNA using electroporation and seeded at 2×10^6 cells/ml into media (CDCHO (Life Technologies), PROCHO5 (Lonza Biowhittiker), MERK (Merck) and PRO/Feed (PROCHO5 and ASF Ajinomoto feed). The cells were cultured for 24 hr at 37° C., and the temperature was then lowered to 32° C. At 72 hr post-transfection, 2 mM sodium butyrate was added, and the cells were cultured for a further 11 days. The mAb expression was determined from the culture supernatant using Protein G HPLC. The data for the relative increase in expression of mAb X from CHOSXE and XE4-1 cells compared to the expression of mAb X from CHOS cells is shown in FIG. 18. The data demonstrates that XE4-1 cells produced increased mAb expression levels of mAb X compared to CHOS cells. The highest levels of mAb X were achieved using PROCHO5 and PROCHO5/Feed media.

Example 10

Growth Characteristics

FIG. 19 indicates the growth characteristics of XE4-1 cells compared to CHOSXE cells. When seeded at a 2×10^5 cells/ml on day 1 and counted again 48 hrs later at day 3, the XE4-1 cells divided slower than the CHOSXE cells. The XE4-1 cell line was sub-cultured for a number of generations to improve its growth characteristics and named XE4-1R.

Example 11

Transfection of XE4-1, XE4-1R and CHOSXE Cells with a Monoclonal Antibody

2×10^8 cells/ml were transfected with 0.4 mg of antibody DNA using electroporation and seeded at 2×10^8 cells/ml into media (CDCHO (Life Technologies)). The cells were cultured for 24 hr at 37° C., and the temperature was then lowered to 32° C. At 72 hr post-transfection, 2 mM sodium butyrate was added, and the cells were cultured for a further 3 days. The supernatant was quantified using Protein G HPLC. FIG. 20 shows transfected XE4-1, XE4-1R and CHOSXE cell numbers. FIG. 21 shows that XE4-1R demonstrated antibody yields (not normalized final titers) equivalent to that of CHOSXE despite their being 2.8-fold less cells at day 6.

It will of course be understood that the present invention has been described by way of example only, is in no way meant to be limiting, and that modifications of detail can be made within the scope of the claims hereinafter. Preferred features of each embodiment of the invention are as for each of the other embodiments mutatis mutandis. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

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Thr His Leu Ser Pro Glu Glu Lys Ala Leu Arg Arg Lys Leu Lys Asn
65     70     75     80
Arg Val Ala Ala Gln Thr Ala Arg Asp Arg Lys Lys Ala Arg Met Ser
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Glu Leu Glu Gln Gln Val Val Asp Leu Glu Glu Asn Gln Lys Leu
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Glu Asn Gln Glu Leu Arg Gln Arg Leu Gly Met Asp Ala Leu Val Ala
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Glu Glu Glu Ala Glu Ala Lys Gly Asn Glu Val Arg Pro Val Ala Gly
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Ser Ala Glu Ser Ala Ala Gly Ala Gly Pro Val Val Thr Pro Pro Glu
165    170    175
His Leu Pro Met Asp Ser Gly Gly Ile Asp Ser Ser Asp Ser Glu Ser
180    185    190
Asp Ile Leu Leu Gly Ile Leu Asp Asn Leu Asp Pro Val Met Phe Phe
195    200    205
Lys Cys Pro Ser Pro Glu Pro Ala Ser Leu Glu Glu Leu Pro Glu Val
210    215    220
Tyr Pro Glu Gly Pro Ser Ser Leu Pro Ala Ser Leu Ser Leu Ser Val
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Gly Thr Ser Ser Ala Lys Leu Glu Ala Ile Asn Glu Leu Ile Arg Phe
245    250    255
Asp His Ile Tyr Thr Lys Pro Leu Val Leu Glu Ile Pro Ser Glu Thr
260    265    270
Glu Ser Gln Ala Asn Val Val Val Lys Ile Glu Glu Ala Pro Leu Ser
275    280    285
Pro Ser Glu Asn Asp His Pro Glu Phe Ile Val Ser Val Lys Glu Glu
290    295    300
Pro Val Glu Asp Asp Leu Val Pro Glu Leu Gly Ile Ser Asn Leu Leu
305    310    315    320
Ser Ser Ser His Cys Pro Lys Pro Ser Ser Cys Leu Leu Asp Ala Tyr
325    330    335
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35          40          45
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50          55          60
Leu Gln Lys Leu Leu Glu Ser Asp Tyr Phe Arg Tyr Tyr Lys Val Asn
65          70          75          80
Leu Lys Arg Pro Cys Pro Phe Trp Asn Asp Ile Ser Gln Cys Gly Arg
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Arg Asp Cys Ala Val Lys Pro Cys Gln Ser Asp Glu Val Pro Asp Gly
100         105         110
Ile Lys Ser Ala Ser Tyr Lys Tyr Ser Glu Glu Ala Asn Asn Leu Ile

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Ser Ser Asp Asn Phe Cys Glu Ala Asp Asp Ile Gln Ser Pro Glu Ala		
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Glu Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys		
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Gly Pro Asp Ala Trp Lys Ile Trp Asn Val Ile Tyr Glu Glu Asn Cys		
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Cys Val Glu Lys Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala		
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Ser Ile Asn Val His Leu Ser Ala Arg Tyr Leu Leu Gln Glu Thr Trp		
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Tyr Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Leu Pro		
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Phe Phe Glu Arg Pro Asp Phe Gln Leu Phe Thr Gly Asn Lys Ile Gln		
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Ser Phe Pro Leu His Phe Asp Glu Thr Ser Phe Phe Ala Gly Asp Lys		
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Lys Glu Ala His Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn		
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What is claimed is:

1. An in vitro recombinant host cell, wherein the cell is from an XE4-1R cell line as deposited at the European Collection of Cell Cultures (ECACC) having the Accession number: 14032501.

2. The cell according to claim 1, wherein the cell further comprises a polynucleotide sequence encoding a protein of interest, wherein the protein of interest is exogenous.

3. The cell according to claim 2, wherein the polynucleotide sequence encoding the protein of interest is within an expression cassette that does not comprise a nucleic acid sequence encoding Endoplasmic Reticulum Oxidoreductin 1 (Ero1), a nucleic acid sequence encoding X-box binding protein 1 (XBP1), or a combination thereof.

4. The cell according to claim 2, wherein the protein of interest is an antibody or an antigen binding fragment thereof.

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5. The cell according to claim 1, wherein the cell is modified to increase the expression levels of a component of the unfolded protein response (UPR) pathway relative to the expression levels of the component of the UPR pathway in an unmodified XE4-1R cell.

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6. The cell according to claim 5, wherein the cell further comprises an exogenous polynucleotide sequence encoding the component of the unfolded protein response (UPR) pathway.

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7. A method of producing a recombinant protein of interest comprising expressing the recombinant protein of interest in a cell of claim 2.

8. A cell as defined in claim 1 for use as a medicament.

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